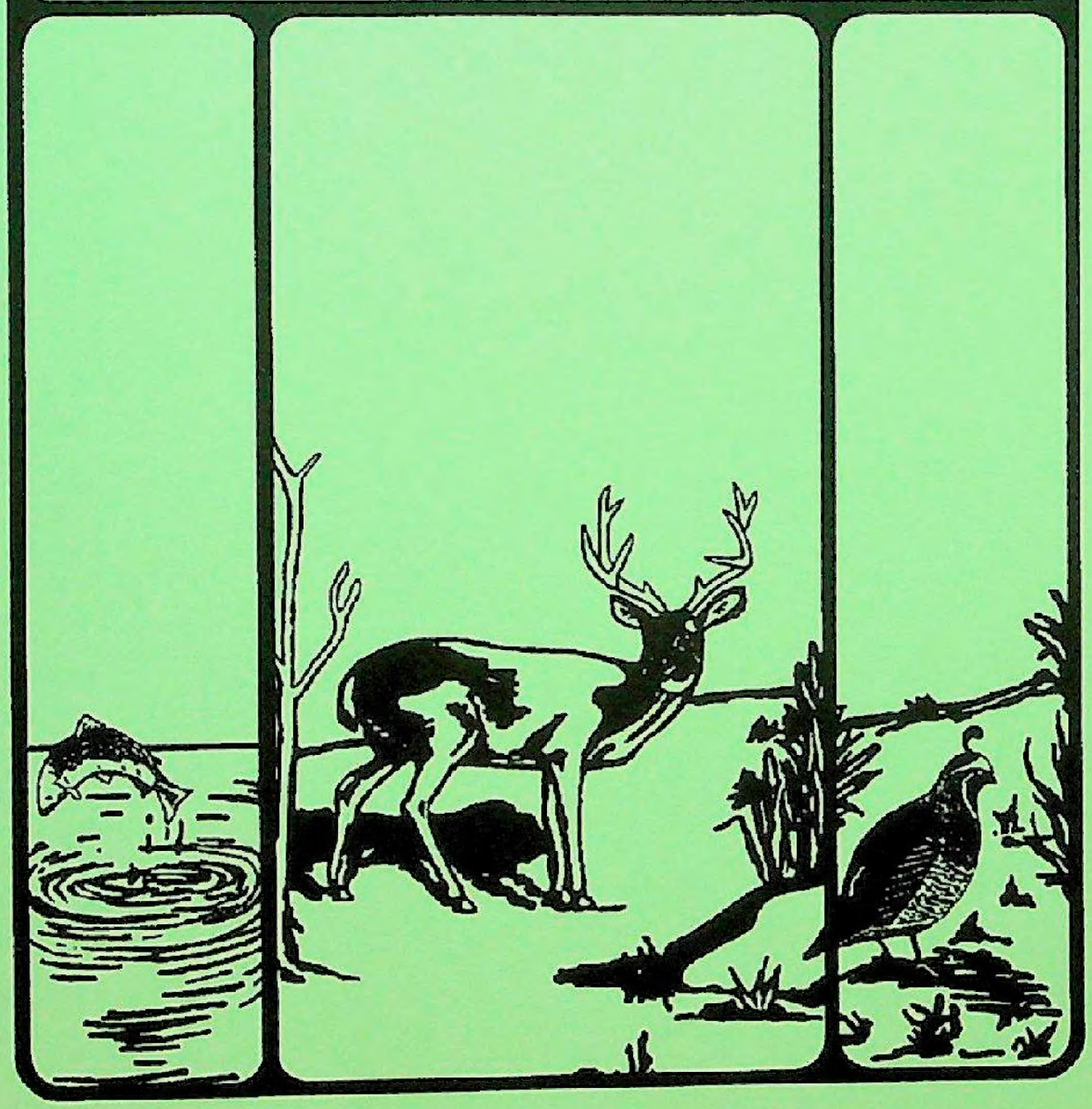
CALIFORNIA FISH AND GAME

"CONSERVATION THROUGH EDUCATION"

VOLUME 93 FALL 2007 NUMBER 4



California Fish and Game is published quarterly by the California Department of Fish and Game. It is a journal devoted to the conservation and understanding of California's flora and fauna. If its contents are reproduced elsewhere, the authors and the California Department of Fish and Game would appreciate being acknowledged.

Subscriptions may be obtained at the rate of $15 ($20 for foreign subscribers) per year by placing an order with the Editor, *California Fish and Game*, P.O. Box 944209, Sacramento, California 94244-2090. Checks or money orders in U.S. dollars should be made out to *California Fish and Game*. Inquiries regarding subscriptions should be directed to the editor. Complimentary subscriptions are granted on an exchange basis.

California Department of Fish and Game employees may request complimentary subscriptions to the journal.

Please direct correspondence to:

Ken Hashagen
Editor-in-Chief
California Fish and Game
11375 Buckeye Hill Ct.
Gold River, CA 95670
e-mail: khashagen@mindspring.com

Alternate communication format is available upon request. If reasonable accommodation is needed, call Ms. Joan Prince at (916) 654-3528 or the California Relay (Telephone) Service for the deaf or hearing-impaired from TDD phones at 1-800-735-2929.

CALIFORNIA FISH AND GAME

VOLUME 93 FALL 2007 NUMBER 4

Published Quarterly by

STATE OF CALIFORNIA
THE RESOURCES AGENCY
DEPARTMENT OF FISH AND GAME
--LDA--

CONTENTS

ARTICLES

NOTES

California Fish and Game 93(4):167-199 2007

A RE-EVALUATION OF AGE, GROWTH, AND BATCH FECUNDITY IN THE CALIFORNIA BARRACUDA, *SPHYRAENA ARGENTEA*, FROM SOUTHERN CALIFORNIA BASED ON SPECIMENS TAKEN FROM 2000 TO 2002

David J. Bottinelli[1] and Larry G. Allen[2]
Nearshore Marine Fish Research Program
Department of Biology
California State University, Northridge
18111 Nordhoff St.
Northridge, CA 91330-8303

ABSTRACT

The purpose of this study was to re-evaluate the age and growth of the California barracuda, *Sphyraena argentea*, previously studied only by the interpretation of scale annuli, and to provide contemporary estimates of batch fecundity. Current data were compared with the results of two previous studies: Walford (1932) and Pinkas (1966). Barracuda were collected from various mainland and island locations in the Southern California Bight from San Diego to Santa Barbara using gillnets and hook and line from April 2000 to October 2002. Subsamples of mature oocytes from preserved ovaries were counted by eye to determine batch fecundity, which was best correlated to fish mass ($R^2 = 0.40$, df = 21, P = 0.001). Batch fecundity, as a function of mass, did not differ significantly from previously reported values (ANCOVA, F = 0.535, df = 1, 31, P = 0.470). For the first time, barracuda were aged using sectioned sagittal otoliths and data were fit to the von Bertalanffy growth equation. Age classes 0 - XVIII were represented and the growth rate was fast during the first year for both sexes. Females were significantly longer than males at every age class (ANCOVA, F = 3.934, df = 1, 402, P = 0.048). A subset of fish was also aged using scale analysis to facilitate comparison to previous studies. Scales were deemed unreliable as aging structures for fish older than 4 years, however, otolith-determined ages were not significantly different from scale ages for age classes 0 to IV (ANCOVA, F = 0.105, df = 1, 75, P = 0.746). The current study collected more young-of-the-year *S. argentea* than historical studies. Additionally, otolith analysis provided greater age class resolution for older fishes, which were previously assigned unreliable ages. Growth rates for age classes I through IV were significantly higher than those reported in both Walford (1932) (t = -10.36, df = 2, P £ 0.05) and Pinkas (1966) (t = -10.08, df = 2, P £ 0.05). We propose that the apparent change in growth rate could be related to the warm/cool regimes of the Pacific Decadal Oscillation.

[1]Current Address – Leon Raymond Hubbard Jr. Marine Fish Hatchery, 4200 Garfield St., Carlsbad, CA 92008

[2]Corresponding Author (larry.allen@csun.edu).

INTRODUCTION

The California barracuda, *Sphyraena argentea* Girard, is a member of the Family Sphyraenidae that is found along the western coast of the United States and Mexico from Southern Baja to Prince William Sound, Alaska (Roedel 1948; Pinkas 1966), including Kodiak Island, Alaska (Ally and Miller 1992; Love 1996). It is the only member of the Sphyraenidae regularly known to inhabit the Southern California Bight (Pinkas 1966). Most commercial and recreational fishing for this species occurs from Point Conception south to San Quintin, Baja California with only sporadic sightings north of Point Conception, except during warmer periods (Pinkas 1966; Schultze 1983). *S. argentea* is a coastal pelagic, schooling species found in open water, often near kelp beds and reefs. However, smaller young fish will enter bays, estuaries and harbors (Pinkas 1966; Allen 1985). All barracuda in the Southern California Bight are likely a single population and most demonstrate a seasonal migration north during the spring and summer months that is well correlated with increasing water temperature (Pinkas 1966; Schultze 1983)

Archaeological evidence shows that this species has been exploited in the southern California area for many centuries by native peoples (Follett 1976). The modern commercial fishery began in California during the late 1800's. The barracuda endured a short period of intense commercial popularity until the banning of purse seines in 1940 and the decreased marketability following World War II. Presently, the recreational fishery accounts for the majority of barracuda landings in southern California. Most of these fish are caught aboard commercial passenger fishing vessels where the species is targeted by most boats from May to September (Pinkas 1966; Schultze 1983).

Two previous management studies have been conducted on this species, both by the California Department of Fish and Game. The first (Walford 1932) discussed the basic life history characteristics of the species. Spawning season was found to occur from April to September, with peak spawning from mid May to mid July. Seasonal records of barracuda larvae abundance from the Southern California Bight generally confirm the findings of Walford (1932) (Moser et al. 2001; Moser et al. 2002). Reproduction in this species was demonstrated to be gonochoric with an almost equal sex ratio, males being slightly more abundant in groups during peak spawning season. Individuals were found to spawn multiple times per year, based on the presence of spent gonads only at the end of the season and both developing and mature oocytes simultaneously in the same fish. Batch fecundity, which correlated best to fish mass, was estimated by counting a subsample of mature oocytes in each ovary. After an aging study (discussed below), it was determined that all females spawn by their third year, and 75% in their second. However, males became sexually mature slightly earlier than females, with all males spawning during their second year (Walford 1932).

Walford (1932) investigated the age and growth of barracuda by counting annuli in the scales located below the pectoral fin in about 7,200 fish. The length-weight data were found to fit the growth equation; $W=.003962*L_t^{2.983}$ where W = weight in grams and L_t = total length in centimeters. The oldest fish examined was thought to be 11 years old, but because of limitations related to the use of scales as aging structures, the

maximum reliable age assigned was 6 years (Walford 1932).

The second major publication on the California barracuda (Pinkas 1966) expanded on the study by Walford (1932) with the inclusion of a tag-recapture study. Barracuda were sampled from the commercial fleet landings and commercial passenger fishing vessels from 1958 to 1961 at various southern California landings and used in length-frequency studies. These fish were again aged using scales with age classes I to IX represented in the survey. However, because it had the smallest estimated variance, only data from 1960 were fit to the widely accepted von Bertalanffy growth equation (Cailliet et al. 1986).

These two historical studies both used scale annuli to age barracuda. Scales, however, while convenient to collect, are not the optimal structure for accurately aging fish. Scales tend to underestimate the age of older fish because annuli near the scale margin are often closely spaced and difficult to interpret (Walford 1932; Lowerre-Barbieri et al. 1994; Secor et al., 1995; Lai et al. 1996). Another problem with the use of scales in age determination is that annuli form over a long period of time. Therefore, the time of year that an annulus forms can vary greatly (Lowerre-Barbieri et al. 1994). Walford (1932) found that annuli formed during May to June for young (age class O and I) barracuda and from June to August for older fish. This difference was attributed to the fact that scale annuli reflect somatic growth. Because smaller fish grow faster, they form an annulus sooner than their slower growing elders. This is also the reason why annuli tend to become crowded and unreadable near the scale margin. Scales are also exposed to the environment and can be lost or damaged during a fish's life and subsequently regenerated, confounding interpretation (Lowerre-Barbieri et al. 1994; Secor et al. 1995a).

The most widely accepted structure for aging in contemporary fishery science is the otolith. Somatic growth processes do not directly influence otolith growth (Wright 1991). This allows the otolith to continue accreting and recording annuli independent of the asymptotic slowing in growth rate as a fish ages (Barbieri et al. 1994). Because they are located in the inner ear, otoliths cannot regenerate, dramatically change shape, or fall off (Secor et al. 1995a). Otolith annuli (opaque bands against a translucent matrix) are usually more reliable than scale annuli in their estimation of the age of older fish and are likely to be formed during the same time each year. Formation of annuli is consistent across many different genera in the same region, and is almost entirely confined to a short period during the spring and summer months. Furthermore, life history events such as spawning season do not seem to affect the formation of annuli (Beckman and Wilson 1995). In comparison to scales, that usually require some degree of subjective interpretation, otoliths tend to be "exceptionally clear, consistent, and easy to interpret" (Lowerre-Barbieri et al. 1994). These qualities make otoliths excellent structures for determining the age of fishes, if the fish can be sacrificed to facilitate removal.

The primary purpose of the present study was to re-evaluate the age and growth of *Sphyraena argentea* using a more reliable aging structure, the otolith. Changes in age and growth parameters as compared to earlier studies were also investigated. Batch fecundity and spawning season, while already extensively discussed by Walford (1932), were also re-examined to determine if significant changes have occurred during

the latter part of the 20th century. Currently, the California barracuda is not a major food fish, however, as stocks of other fishes decline, commercial fishing for the species is likely to expand. Re-evaluation of basic life history traits of commercially and recreationally important species is essential information for the proper management of such resources. These analyses are especially needed as lawmakers will be confronted by environmental changes and increased fishing pressure driven by an ever-increasing world population. The Marine Life Management Act (MLMA), enacted in 1999, indeed recognizes that California's ocean resources cannot be protected by static regulations and that preemptive action may be necessary to ensure sustainability. This study will provide fisheries managers with more accurate data that could be used, in conjunction with historical data, to draft a Fisheries Management Plan for *Sphyraena argentea* under the MLMA, if necessary.

MATERIALS AND METHODS

Collection of specimens

A total of 638 *Sphyraena argentea* was collected from 2000-2002 during the months of April to October (Table 1). Most individuals (n =421) were collected as part of the by-catch associated with the ongoing white seabass monitoring program conducted by California State University on behalf of the California Department of Fish and Game.

Table 1. Source of specimens collected for this study. Seven individuals without recorded date or location data are not included in the table. Eight juvenile fish collected in San Diego Bay in 1997 are also not represented.

Site	April	June	July	August	October	Total
Santa Catalina Island	49	185		38	62	334
Palos Verdes	25	33		51	27	136
Seal Beach		17		11		28
Newport Bay	1	24		2		27
Marina del Rey		19		4		23
Malibu	7	6			7	20
Santa Barbara		2			17	19
San Pedro			17			17
Huntington Beach			8			8
Santa Cruz Island				7		7
San Clemente Island				6		6
San Diego Bay				5		5
Ventura					1	1
Total	82	286	25	124	114	631

Barracuda were sampled throughout southern California aboard the *R/V Yellowfin* and *R/V Vantuna* at the following locations: Newport Bay, Seal Beach, Marina del Rey, Palos Verdes, Malibu, Ventura, Santa Barbara, Santa Catalina Island, Santa Cruz Island, and San Clemente Island (Fig. 1). The sampling gear consisted of monofilament gillnets approximately 45 meters long and 2.5 meters high. Each net consisted of six panels with mesh sizes of 2.5, 3.8 or 5 cm. These nets were set near shore, in close proximity to kelp beds during the late afternoon, allowed to fish through the night, and retrieved early the next morning. After capture, specimens were dissected aboard the research vessel or placed on ice and/or frozen for later workup in the lab.

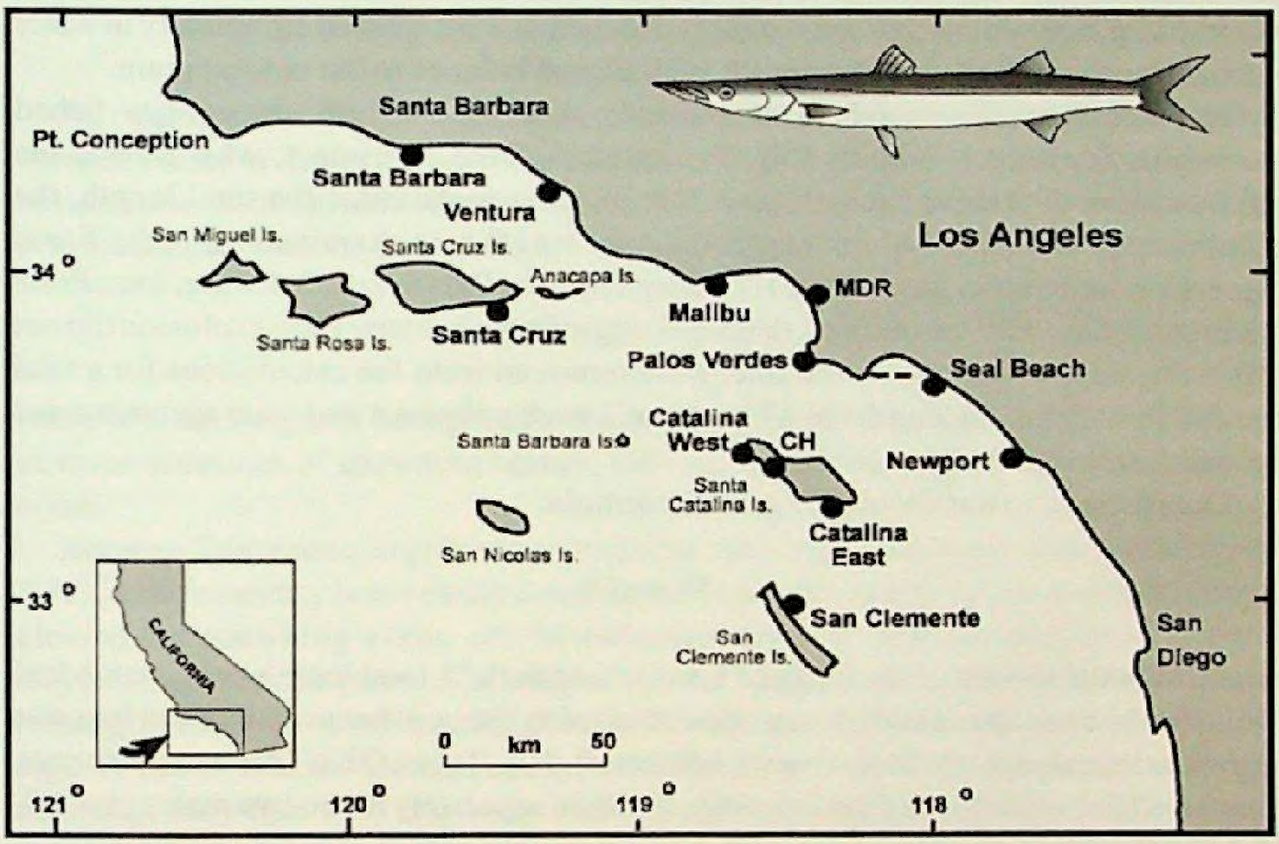


Figure 1. General map of collection area. Black circles indicate gillnetting sites.

Additional samples (n = 136) were collected aboard the commercial passenger fishing vessels *Pursuit* and *Monte Carlo*, which operate out of San Pedro, CA. Fish were collected aboard these boats on three non-consecutive days in two general locations: Santa Catalina Island and Huntington Beach. The patrons aboard the vessels caught barracuda exclusively on hook and line and most allowed biological data to be gathered. Specimens were weighed, measured, and decapitated to allow later removal of otoliths in the lab. A small number of fish (n = 74) were collected opportunistically using hook and line at the following locations: San Diego Bay, Newport Bay, Palos Verdes, San Pedro, Santa Catalina Island and Santa Cruz Island.

Eight juvenile barracuda collected as part of an unrelated study in San Diego Bay (Allen et al. 2002) were also included in some calculations due to their small size and the lack of juvenile fish from other collections. These individuals were captured using

a purse seine in 1997. Seven other individuals were collected without date, location or gear data and were only included in analyses that did not depend on such values.

Length-Weight Relationship

Each fish was measured to the nearest millimeter and weighed. Fish length was expressed in both standard length (tip of snout to end of hypural plate) and total length (tip of snout to tip of caudal fin). This was done in order to compare current findings to the two previous studies that measured only total length. When fish mass was determined in the field, a 5 kg Pesola spring scale was used and mass recorded to the nearest 25 g. Specimens that were returned to the lab were thawed completely in water if frozen and weighed on a Mettler PE3000 digital balance to the nearest gram.

In some cases, barracuda were partially consumed in gillnets as they fished overnight. Scavengers usually left the skeleton of the fish intact, which made the measurement of standard length possible. In order to estimate the total length, the relationship between standard length and total length was examined using the linear regression function in the statistical package Systat v9.0 (Systat Software, Inc., Point Richmond, CA). Eleven outliers, deemed insignificant because their exclusion did not affect the slope of the regression line, were removed from the calculations for a total n = 583 fish. The total lengths of 47 barracuda were unknown and were reconstructed by using this regression equation.

Data were fit to the allometric growth formula:

$$W = aL^b$$

where W = the weight of the fish and L = the length (L_T = total length or L_S = standard length). The constants a and b were calculated using the non-linear estimation function in the statistical package Statistica v6.1 (Statsoft, Inc., Tulsa, OK). This was performed first for all fish regardless of sex (n = 640), and then separately for known males (n = 203) and females (n = 216). To compare the length-weight curves of male and female fish, data were first log-transformed ($\log_{10}(x)$) to ensure normality and homoscedasticity of the dataset. Analysis of covariance (ANCOVA) was subsequently employed to discern any statistical difference in the slope or magnitude of the regression lines.

Calculations were run initially with fish length expressed in mm standard length and weight in grams. However, in order to directly compare this study to Walford (1932), the analysis was repeated using cm total length. The comparison to previous studies was confounded because the raw data from Walford (1932) were not available for comparison using ANCOVA. Instead, a comparison of regression slopes using a t-test was employed. Data from the current study (expressed in cm total length) were log-transformed ($\log_{10}(x)$) and analyzed in Systat v9.0 using the linear regression function. The Walford (1932) study included very few fish under 300 mm and the eight juvenile barracuda from San Diego Bay greatly distorted the regression line for the current study. Therefore, these eight fish were excluded from the analysis along with five other statistically insignificant outliers that did not affect the slope of the regression line (n

= 627). As raw data were unavailable from Walford (1932), the exponent b from the published allometric growth equation, which should approximate the regression slope of the original data, was used in its place. The test statistic (t) was generated using the equation:

$$t = (b_b - b_w)/ SE_b$$

where b_b = slope from the present analyis, b_w = slope from the Walford (1932) study, and SE_b = the standard error from the present analysis.

Spawning Season and Batch Fecundity Estimate

All ovaries collected (n = 183), regardless of whether they were removed aboard the research vessel or dissected later were frozen. Ovaries were later thawed, weighed to the nearest tenth of a gram and preserved in 10% Formalin. Gonosomatic indices (GSI) were calculated with the formula:

$$GSI = (W/B)*100$$

where W is the weight of both gonads and B is the weight of the fish. In order to confirm previous estimates of spawning season, GSI values were averaged and examined by month.

Because *Sphyraena argentea* has multiple spawning events per season (Walford 1932), total fecundity is not easily determined. Batch fecundity, or the number of eggs released per spawning event, can be estimated by counting the number of mature oocytes in a particular gonad (Calliet et al. 1986). As gonads were not examined before freezing or preservation in Formalin, detailed information about the state or developmental stage is not available. Consequently, in order to get a reliable estimate of batch fecundity, only fish with relatively high gonosomatic indices (GSI > 6.0) captured during the peak spawning season (mid-May to mid-July) were included in the batch fecundity analyses (n =23). This value was chosen arbitrarily after examination of the gonosomatic indices of 183 individuals, where 6.0 was a relatively high value. This raised the probability that only fish about to release eggs would be included in the batch fecundity estimate.

Mature oocyte counts were made in a manner similar both to Walford (1932), and the gravimetric method described in Cailliet et al. (1986). One lobe of the ovary (randomly chosen, except in cases where one of the lobes was ruptured) was blotted dry and weighed to the nearest tenth of a gram on a Mettler PE3000 digital balance. Three cross sectional wafers of approximately equal thickness (~ 5mm) were cut from consistent locations in the ovary. The first was cut from the center of the ovary. The two remaining sections were taken from center of the posterior and anterior halves of the ovary. These sections were then weighed to the nearest tenth of a gram.

Preservation in formalin transformed the once flexible ovary into a semi-rigid structure in which the oocytes became opaque and hard. While this new rigidity was

good for the cutting of cross sections and oocyte counting, separation of oocytes from ovarian tissue became difficult. Oocytes could be gently separated from the surrounding tissues by rubbing the sections against a nylon mesh that was submerged in fresh water. The surrounding tissue disintegrated and the hardened oocytes remained intact. Due to preservation in formalin, oocytes were assumed to have lost some of their original size. Oocytes did not appear to have ruptured from freezing although they were no longer perfectly spherical. Walford (1932) conducted a thorough examination of barracuda oocytes and found three distinct size classes: immature (< 0.2mm), maturing (0.2 to 1.04 mm) and mature (1.24 to 1.6 mm). Oocytes were transferred to a Petri dish, spread about evenly, and examined over a black background with white gridlines. The mature oocytes (those which will be released in the next spawning event) were counted by eye. As there was a noticeable and obvious difference in the relative diameters of mature oocytes and the other two size classes, measuring of individual oocytes was not deemed necessary. Thus, the number of mature oocytes per gram of preserved ovary could be determined and then extrapolated to represent the whole gonad.

The relationship between batch fecundity and three factors, fish age, ovary-free fish mass (Hunter and Goldberg 1980), and fish length (mm total length), was investigated. Data were log-transformed ($\log_{10}(x)$) for the same reasons mentioned earlier, and analyzed using the linear regression function in Systat v9.0. Historical batch fecundity data were available for eleven fish (Walford 1932, Table 2) and were analyzed in the same manner. ANCOVA was used to determine if batch fecundity estimates differed significantly from the current results. First, the slopes of the regression lines were tested for homogeneity. Once this was confirmed, the elevation of each regression slope was examined to discern any significant difference in magnitude.

The determination of age or size at first maturity was extensively examined by Walford (1932), in fact such estimates are referenced in this study. The fact that gonad samples were not examined before freezing or fixing in Formalin, prevented a reassessment of this aspect of the species life history. Additionally, no histological sections were collected, as the primary concern was obtaining a batch fecundity estimate.

Age Determination from Otoliths

Otoliths were removed using a method developed and employed by Allen et al. (1995) and first described by Craig et al. (1999). First, the parasphenoid bone of the fish was exposed by placing the fish on its dorsal side and removing the flesh and dorsal portion of the gill arches. A thin groove was then cut in the parasphenoid using a serrated knife or small coping saw to facilitate fracture perpendicular to the long axis of the bone. Once broken, sagittal otoliths were easily located, removed with forceps, cleaned, dried, and stored in plastic microcentrifuge tubes. In most cases, the left sagitta was used for aging. However, in cases where the left otolith was missing, cracked, or otherwise damaged, the right was substituted. In the laboratory, the widest part of the left sagitta was measured to the nearest hundredth of a millimeter using digital calipers. The otolith was then weighed to the nearest ten-thousandth of a gram on a Mettler AM50 analytical balance.

Table 2: Morphometric, age and extrapolated mature oocyte counts used in batch fecundity calculations.

Sample number	Body mass (g)	Ovary mass (g)	Gonad-free mass (g)	Age (years)	Total length (mm)	Batch Fecundity
1	1851	110	1741	7	837	183751
2	2840	182	2658	14	955	286391
3	1489	127	1362	8	860	211939
4	2090	131	1959	9	810	279351
5	1354	88	1267	2	742	177346
6	1751	210	1542	5	821	201942
7	1675	136	1539	3	747	244465
8	1700	104	1596	3	802	193171
9	2075	134	1941	7	882	221470
10	2100	151	1949	7	850	271968
11	2600	172	2428	10	901	369093
12	2075	138	1937	6	870	274911
13	2500	250	2250	11	863	469160
14	2000	203	1797	11	830	397294
15	2075	185	1890	8	803	378457
16	2050	182	1868	10	800	426930
17	1400	106	1294	2	720	218491
18	2400	207	2193	13	865	357134
19	2550	178	2372	10	849	332055
20	2250	280	1970	6	808	512987
21	1750	166	1584	3	740	288743
22	1250	95	1155	2	700	202991
23	1850	168	1682	5	781	335272

In preparation for sectioning, cyanoacrylate (super glue) was used to attach each otolith to a small wood block. The block was then mounted in a Buehler Isomet low speed saw, equipped with two diamond edged blades spaced 0.5 mm apart. The section was then removed from the wood block, polished with silicon carbide lapping paper and placed in a water-filled, black-bottomed watch glass (Allen et al. 1995). Each otolith was read under a dissecting microscope once and later read again by the same person. The second examination was performed randomly months later, and was independent of the first read. If the two values did not agree, a third reading was taken to determine the best age estimate. Percent agreement was easily computed as the number of agreements between the first and second read, divided by the total and multiplied by 100. Percent agreement was again calculated after the third reading.

The otoliths of *Sphyraena argentea* are long, thin and relatively small, which is characteristic of a fast moving pelagic species (Secor et al. 1991). The anterior end of the otolith has a pronounced point and the posterior end is rounded. In young fish, the otolith was thin enough at the margins to feasibly allow the counting of annuli without sectioning, however all data was collected as described above. Otoliths of young fish

(Fig. 2a) were noticeably smoother on the margins than those of older individuals, which tended to have more pronounced bumps and irregularities (Fig. 2b).

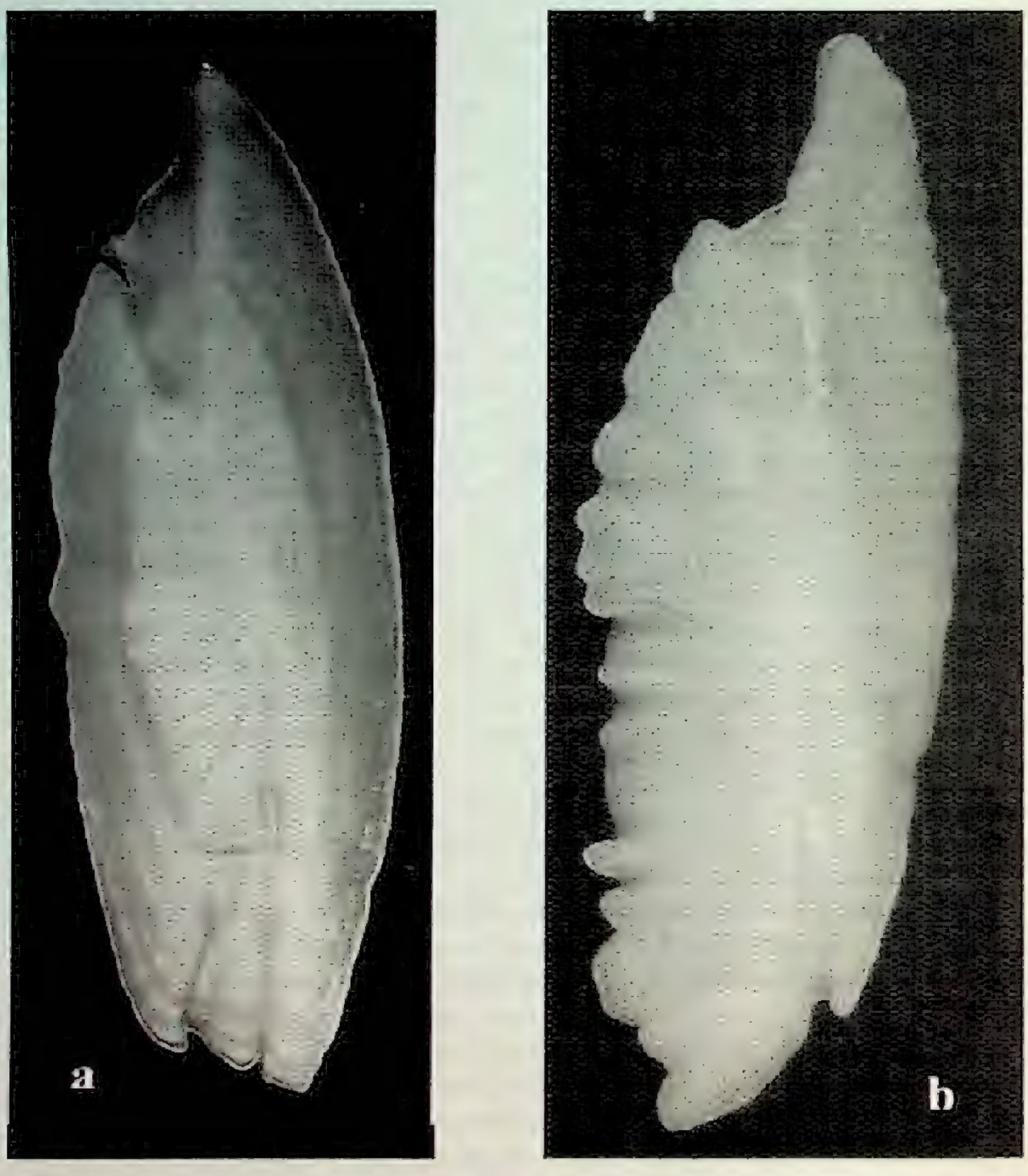


Figure 2. (a) The relatively smooth sagittal otolith of a young (one year old) *Sphyraena argentea* (width = 3.74mm) (b) The sagittal otolith of an 18 year old fish (width = 5.09mm).

After sectioning, sagitta proved to be extremely easy to read under a dissecting microscope (Fig. 3). In total, 600 otoliths were successfully aged, 9 were removed from the data set due to confounding third reads, and 37 were lost or irrevocably damaged during removal or preparation. Otoliths that were difficult to read were polished with

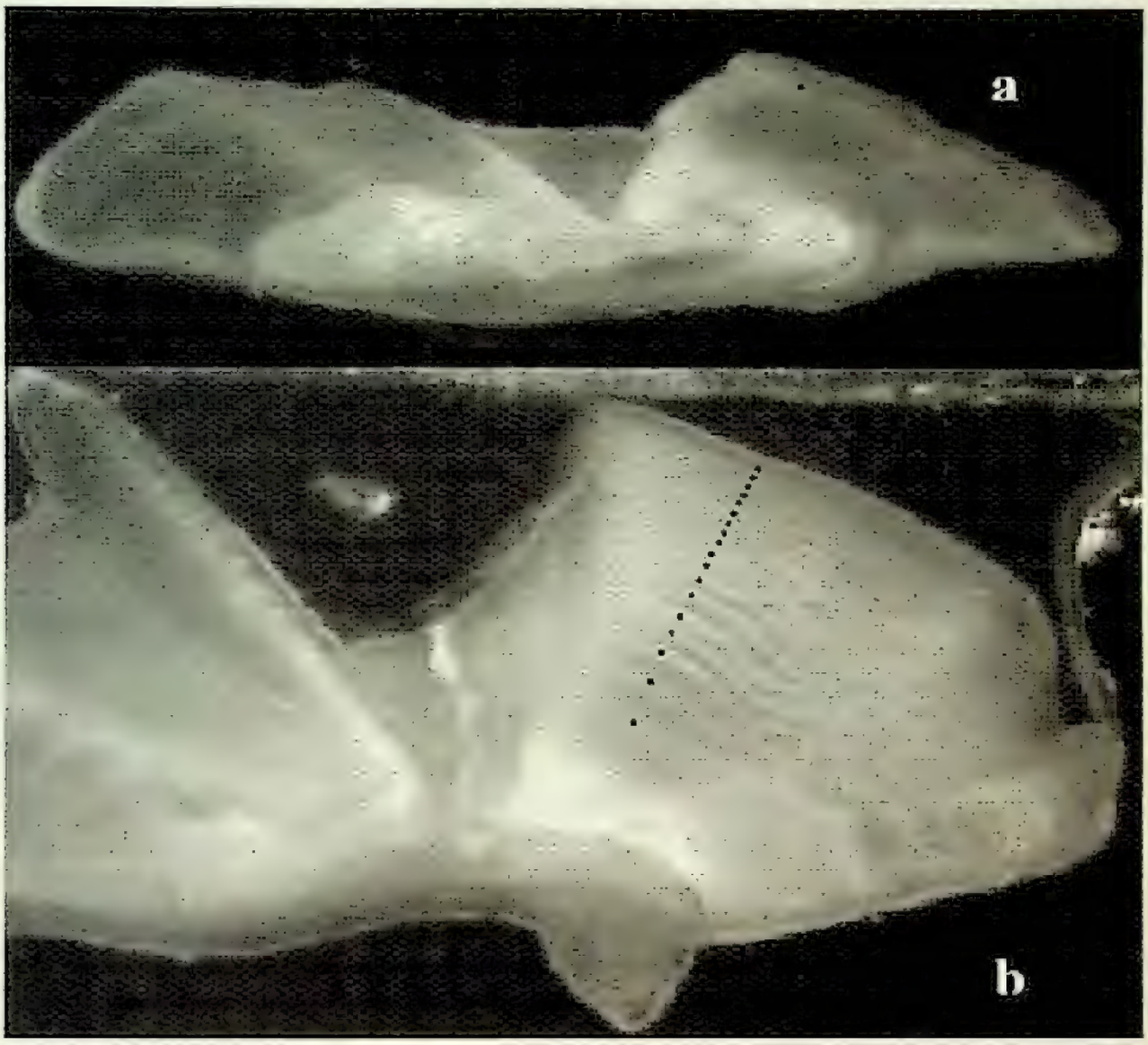


Figure 3. The sectioned sagittal otolith of a one-year-old fish (a) and an eighteen-year-old fish (b) shown under a dissecting microscope. Black dots indicate annuli.

silicon carbide lapping paper until the annuli were clearly visible, however, extensive polishing was rarely necessary.

The relationship between otolith morphometrics and age was examined using linear regression. Otolith mass and width were log-transformed ($\log_{10}(x+1)$) and plotted against otolith-determined age using Systat v9.0. $\text{Log}_{10}(x+1)$ was used to eliminate the negative infinity problem associated with $\log_{10}(0)$. Two outliers that did not significantly affect the regression slope were excluded from the otolith mass calculations (n=530). Otolith width calculations were based on n=592 after the exclusion of eight outliers that did not affect the regression line. The results of these analyses were used to validate the annual nature of otolith annuli in *Sphyraena argentea*.

FishParm v3.0S (Elsevier Scientific Publishers Co., Bronxville, NY) was used to fit length-at-age data to the von Bertalanffy growth model:

$$L_t = L_\infty(1 - e^{-k(t-t_0)})$$

where L_t = length at time t, L_∞ = predicted maximum length, k = constant growth coefficient, t_0 = theoretical size at age zero and e = the base of the natural log (2.718) (Cailliet et al. 1986).. The mean lengths of 19 age classes comprised of 596 individuals were included in the analysis. The sample included male and female barracuda, as well as immature fish, and those that could not be sexed. Data from males and females was log-transformed and used to generate linear regression lines using Systat v9.0. Differences in length-at-age between males (n = 196) and females (n = 209) were examined using the analysis of covariance (ANCOVA) function in Systat v9.0. First the slopes of the regression lines were tested for homogeneity. When this was confirmed, the elevation of each regression slope was examined to discern any significant difference in magnitude.

The length-at-age data for the current study was compared to published results from Walford (1932) and Pinkas (1966). The von Bertalanffy parameters for the current data had to be recalculated using total length measurements in order to compare them to these previous studies. Mean lengths-at-age and their corresponding standard deviations were extracted from a graph provided by Walford (1932, Fig. 25) and entered into FishParm v3.0s to generate von Bertalanffy parameters for age classes I-VI. The von Bertalanffy parameters for the Pinkas (1966) study, based on data from one year (1960) and composed of age classes I through VIII, were L_∞ = 1022.54 mm total length, k = .249 and t_0 = -.769; however, the actual mean length-at-age is unknown.

Again, because the raw data were not available from the previous studies, a t-test was used to compare the slopes of each length-at-age regression line. The actual mean length-at-age data from this study was log-transformed and regressed on the log-transformed age data using Systat v9.0. Only age classes I through IV were used because of the uncertainty of age determination based on scale annuli in older individuals. Results of an ANCOVA (discussed below) justify this comparison. In the same manner, a regression slope for the Walford (1932) data was created using actual mean lengths for age classes I through IV. The slope of the regression based on Pinkas (1966) data could not be based on actual mean lengths-at-age, as these were unknown. Out of necessity, lengths-at-age were instead calculated using theoretical mean lengths computed from the published von Bertalanffy parameters. The test statistic (t) was generated using the equation:

$$t = (b_b - b_x)/SE_b$$

where b_b = slope of Bottinelli-Allen regression line, b_x = slope of Walford or Pinkas regression line, and SE_b = the standard error of Bottinelli-Allen regression. The hypothesis tested in both comparisons was Ho: $b_b = b_x$.

Age Determination from Scales

The primary focus of this study was age determination using sagittal otoliths. However, the congruence of otolith and scale-derived ages has never been examined

in this species, and such information will be useful when discussing previous studies. Scales were collected from under the left pectoral fin (Walford 1932; Pinkas 1966) and stored in seawater-filled microcentrifuge tubes. Using the ages previously determined by otolith examination, a maximum of 10 fish from each age class (when available) were selected at random for study (n = 60). Three scales from each fish were mounted in water on a glass slide and examined under a compound microscope. The same reader later read the same three scales for a second time. When age estimates disagreed, a third read was made in the manner described above. Length-at-age data for age classes I through IV from both the scale and otolith analyses and their corresponding total lengths were analyzed using linear regression. ANCOVA was used to determine if the scale-derived regression line differed significantly from the line fitted to otolith data in either slope or magnitude.

RESULTS

Length-weight Relationship

A total of 638 barracuda was collected for this study ranging from 305 to 820 mm standard length (Fig. 4). The additional eight individuals collected in 1997 from San Diego Bay (Allen et al. 2002) ranged from 105 to 180 mm standard length.

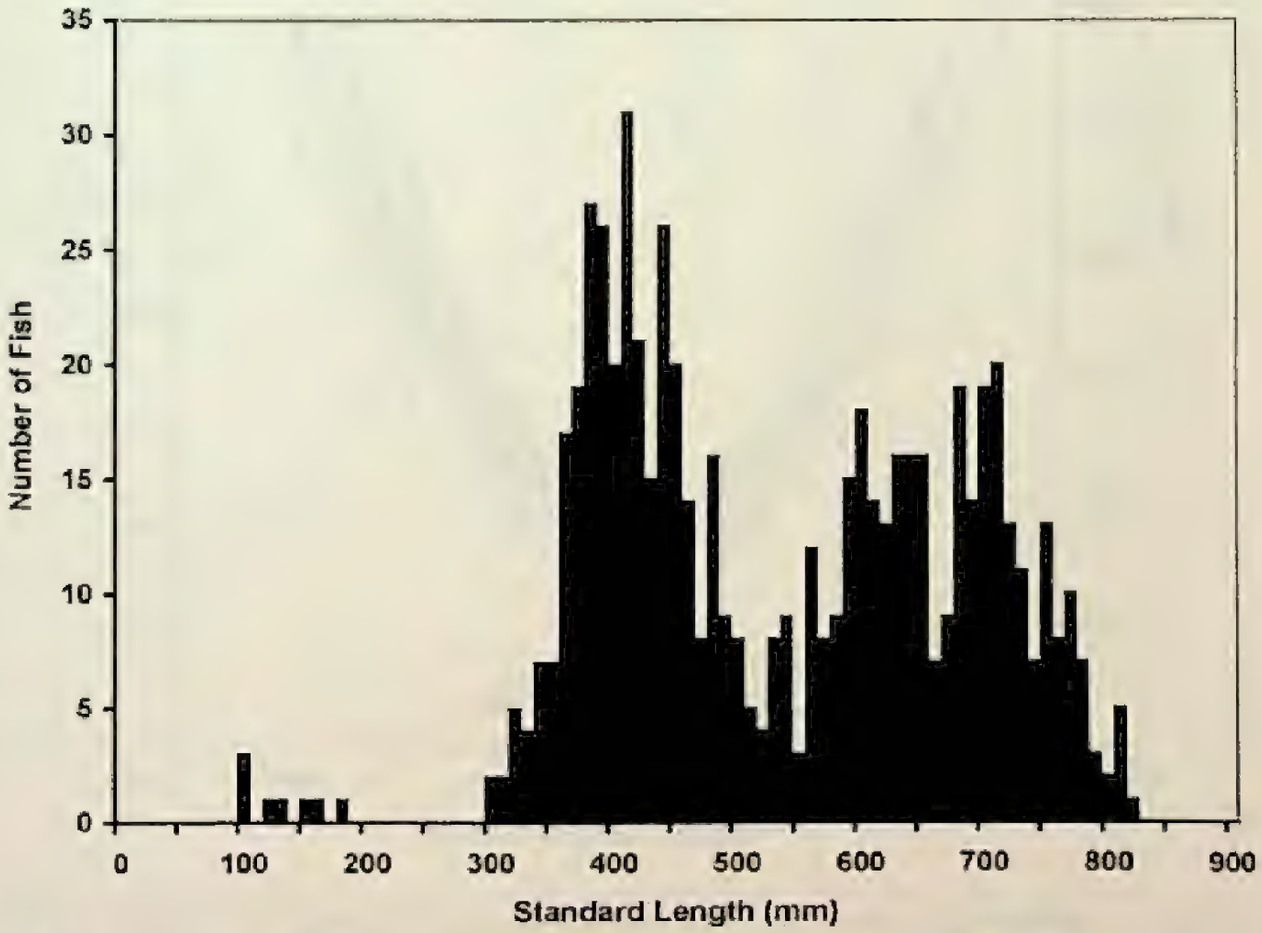


Figure 4. The length frequency of all specimens collected for study compiled into 10 mm bins (n = 646).

The relationship between total length and standard length was best described by the equation $y = 1.148x + 10.744$, where y is the total length (mm) and x is the standard length (mm). This was determined by a significant linear regression ($R^2 = 0.99$, $df = 581$, $P < 0.001$). This equation was subsequently employed to compute the unknown total lengths of a small number of fish in order to compare them to previous studies.

The relationship between standard length (L_S) and weight (W) in *Sphyraena argentea* was best described by the significant equation: $W = 0.000028 * L_S^{2.754}$ ($R^2 = 0.95$, $df = 638$, $P < 0.001$) (Fig. 5). In order to compare this relationship to the Walford (1932) treatment, length data was converted from standard length (mm) to total length (cm). This total length-weight curve is best described by the equation: $W = 0.007856 * L_T^{2.818}$ ($R^2 = 0.95$, $df = 638$, $P < 0.001$).

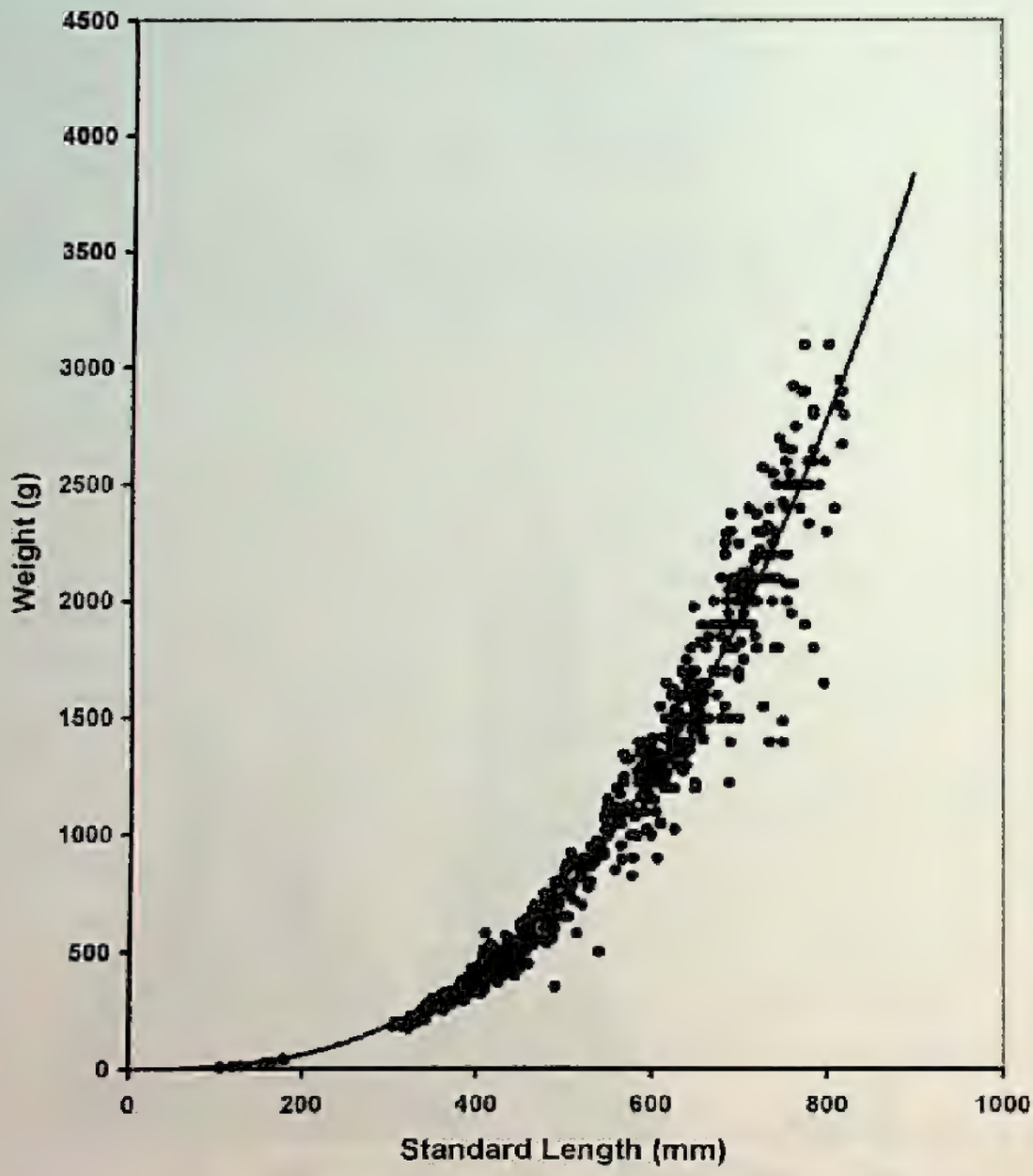


Figure 5. Length-weight curve defined by the allometric growth equation $W = 0.000028 * L_S^{2.754}$ where W = the weight of the fish in grams and L_S = the standard length of the fish in millimeters ($R^2 = 0.9522$, $df = 638$, $P < 0.001$).

When fish of known sex were calculated separately, the equation $W = 0.000009 * L_S^{2.943}$ was found to fit the data for male fish well ($R^2 = 0.96$, df = 201, $P < 0.001$) and $W = 0.000019 * L_S^{2.808}$ best described the females ($R^2 = 0.96$, df = 214, $P < 0.001$) (Fig. 6). Linear regression of log-transformed data for male fish fit the line $y = 2.961x - 5.1173$, where y is the $\log_{10}$ of fish mass (g) and x is the $\log_{10}$ of standard length (mm) ($R^2 = 0.97$, df = 201, $P < 0.001$). Female fish were also well described by the significant linear equation $y = 2.8367x - 4.7969$ ($R^2 = 0.9787$, df = 214, $P < 0.001$). The slight difference seen in the slopes of the two regression lines was found to be significant (ANCOVA, $F = 7.85$, df = 1, 415, $P = 0.005$).

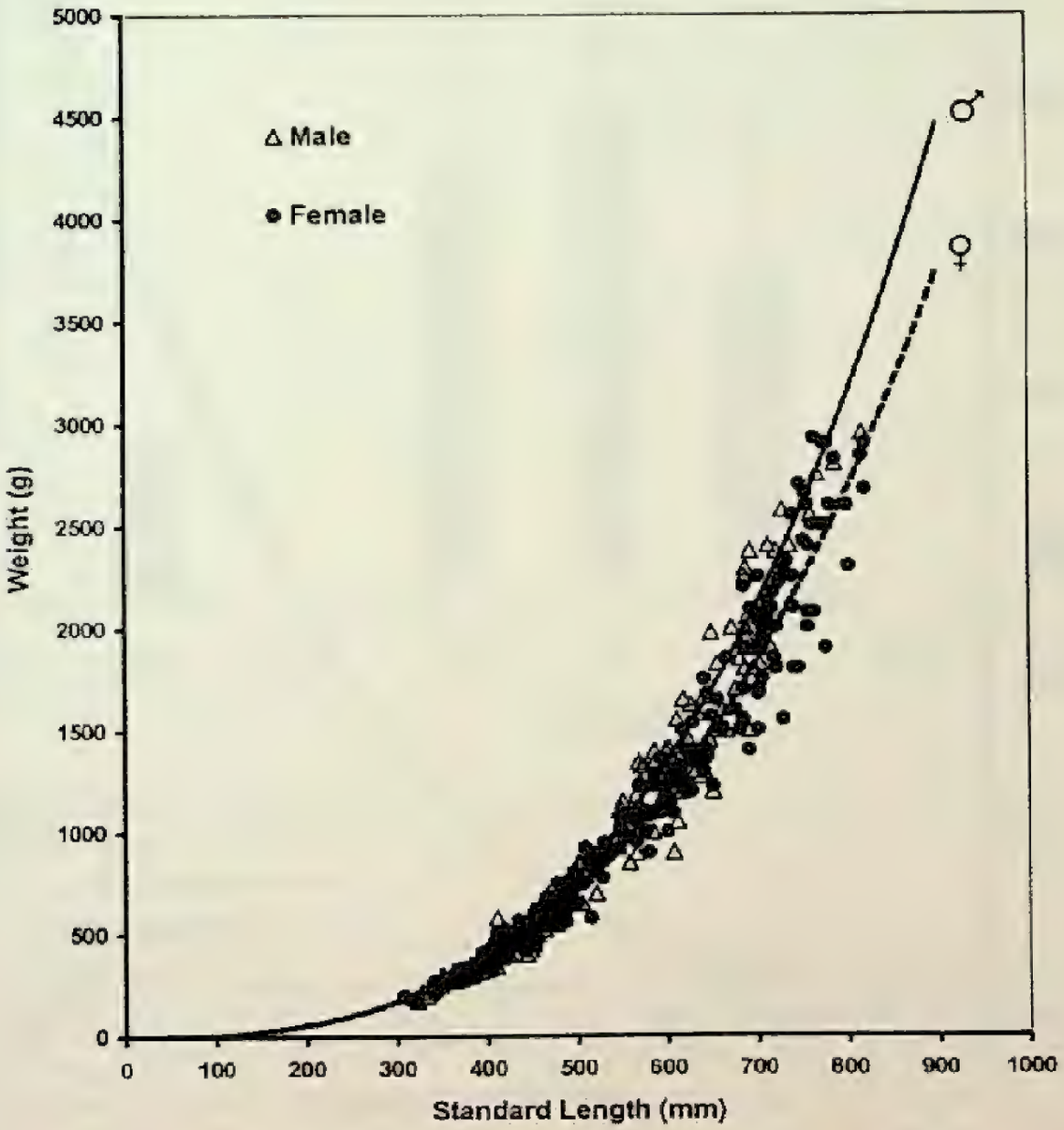


Figure 6. Length-weight curves for fish of known sex. The male curve is defined by the allometric growth equation $W = 0.000009^* L_S^{2.943348}$ where W = the weight of the fish in grams and L_S = the standard length of the fish in millimeters ($R^2 = 0.96$, df = 201, $P < 0.001$). The equation $W = 0.000019^* L_S^{2.807810}$ defines the curve for female fish ($R^2 = 0.96$, df = 214, $P < 0.001$).

The length-weight curve for Walford (1932) is based on values generated from the published equation $W = 0.003962 * L_T^{2.983}$ and the Bottinelli-Allen curve, after conversion to total length, is defined by $W = 0.007856 * L_T^{2.818}$ (Fig. 7). The significant regression equation for the current study, based on log-transformed values, is $y = 2.9311x - 2.3153$ ($R^2 = 0.98$, df = 625, $P < 0.001$). The slope from the Walford study was significantly different ($t = -3.058$ df = 625, $p \leq 0.05$).

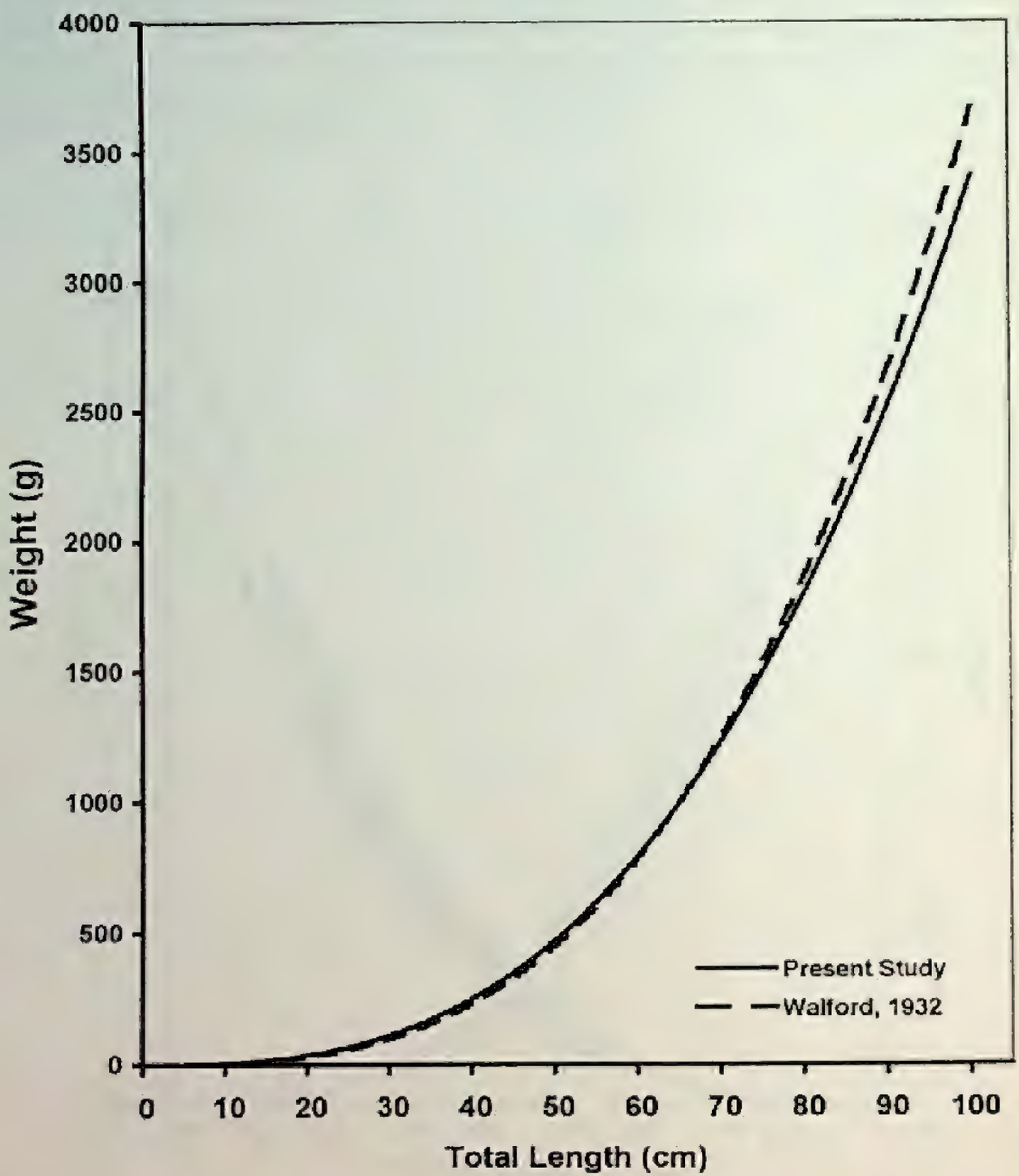


Figure 7. Length-weight curves for Walford (1932) and the current study. The Bottinelli-Allen curve is defined by the allometric growth equation $W = 0.007856 * L_T^{2.818}$ where W = the weight of the fish in grams and L_T = the total length of the fish in centimeters ($R^2 = 0.9530$, df = 638, $P < 0.001$). The Walford line is defined by the published equation $W = .003962 * L_T^{2.983}$.

Spawning Season and Batch Fecundity Estimate

Mean monthly gonosomatic index (GSI) for female fish (n = 183, standard length 318 – 818 mm) was highest in June (GSI = 4.82) and July (GSI = 5.55). Standard deviation around the mean monthly GSI was higher during the peak spawning season (mid-May to mid-July) and lowest in April and October (Fig. 8).

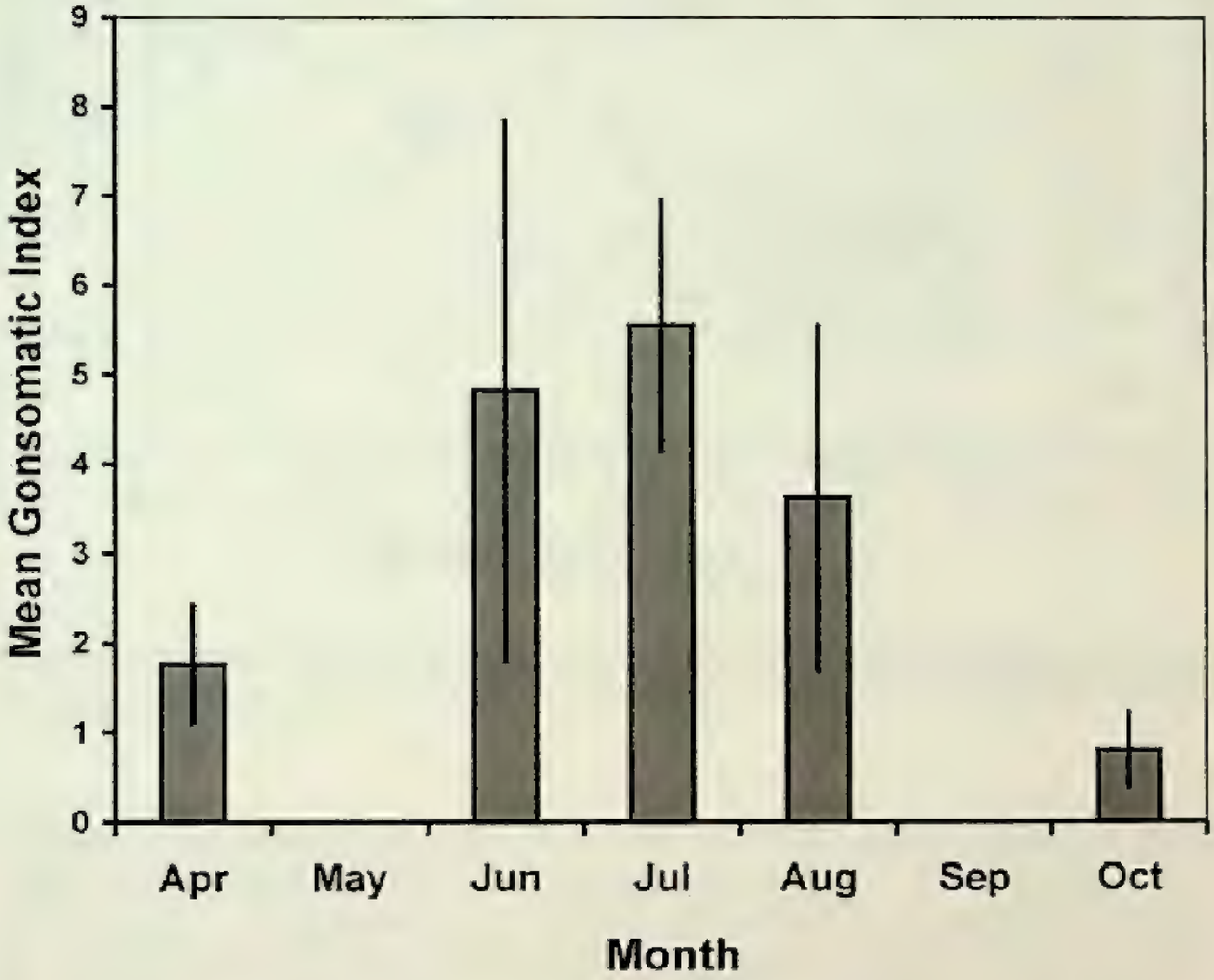


Figure 8. Mean gonosomatic index (GSI) for female fish (n = 183). Error bars indicate ± one standard deviation.

The number of mature oocytes in each gonad (Table 2) was significantly related to the weight of the fish (Fig. 9) and was defined by the equation $y = 0.9136x + 2.4815$ ($R^2 = 0.40$, df = 21, P = 0.001), where y is the $\log_{10}$ of the number of mature oocytes and x is the $\log_{10}$ of mass (g) . Regression of log-transformed ages (age classes II to XIV) and the number of mature oocytes produced the line $y = 0.3061x + 5.2138$ ($R^2 = 0.3615$, df = 21, P = 0.002). The third factor, total length (mm), was not a significant predictor of batch fecundity ($R^2 = 0.0827$, df = 21, P = 0.183).

Recalculation of the data presented in Walford (1932) yielded a significant relationship between batch fecundity and mass that fit the line $y = 1.0604x + 1.9756$ (R^2

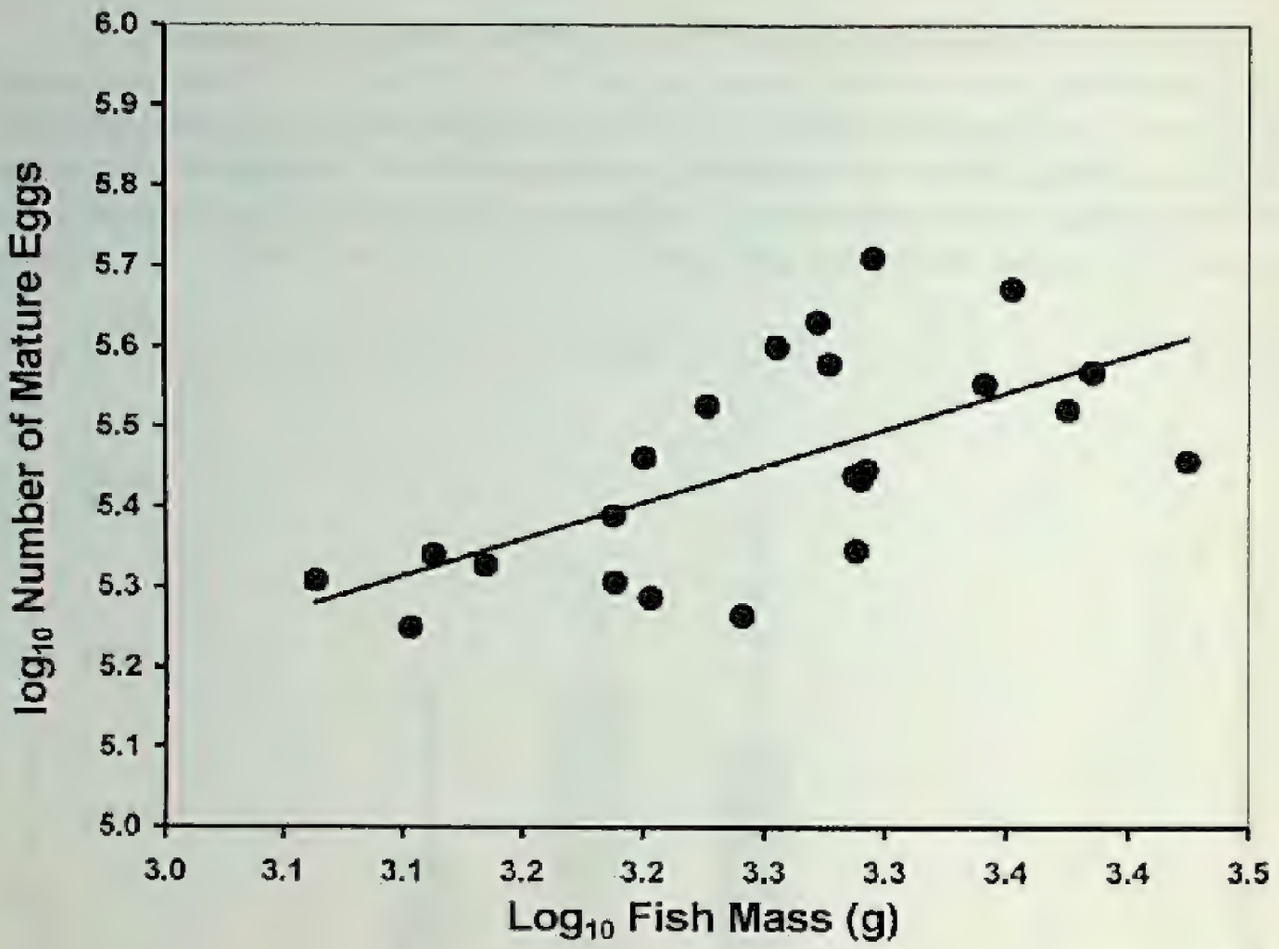


Figure 9. Linear regression line based on the log-transformed values for mass and the number of mature oocytes (R^2 = 0.40, df = 21, P = 0.001).

= 0.8879, df = 9, P < 0.001), where y is the $\log_{10}$ of the number of mature oocytes and x is the $\log_{10}$ of mass (g). These results were not significantly different from this study in slope (ANCOVA, F = 0.273, df = 1, 30, P = 0.605) or magnitude (ANCOVA, F = 0.535, df = 1, 31, P = 0.470) (Fig. 10). Age was also significantly related to batch fecundity and was defined by y = 1.6299x + 4.1986 (R^2 = 0.8785, df = 9, P < 0.001), where y is the $\log_{10}$ of the number of mature oocytes and x is the $\log_{10}$ of age (years). However, the regression slope differed significantly from the current study (ANCOVA, F = 41.461, df = 1, 30, P = < 0.001), which prevented further analysis of slope magnitude (Sokal and Rolf 1995). Batch fecundity as a function of total length, based on log-transformed values was significantly described by the line y = 3.3011x - 4.2041 (R^2 = 0.9004, df = 9, P < 0.001) but was also found to differ significantly in slope from the current study (ANCOVA, F = 5.209, df = 1, 30, P = 0.03), that barred analysis of slope magnitude.

Age Determination from Otoliths

Age zero fish accounted for 30.5% of all samples examined. The oldest fish encountered in this study was 18 years of age (Fig. 11). Percent agreement between the

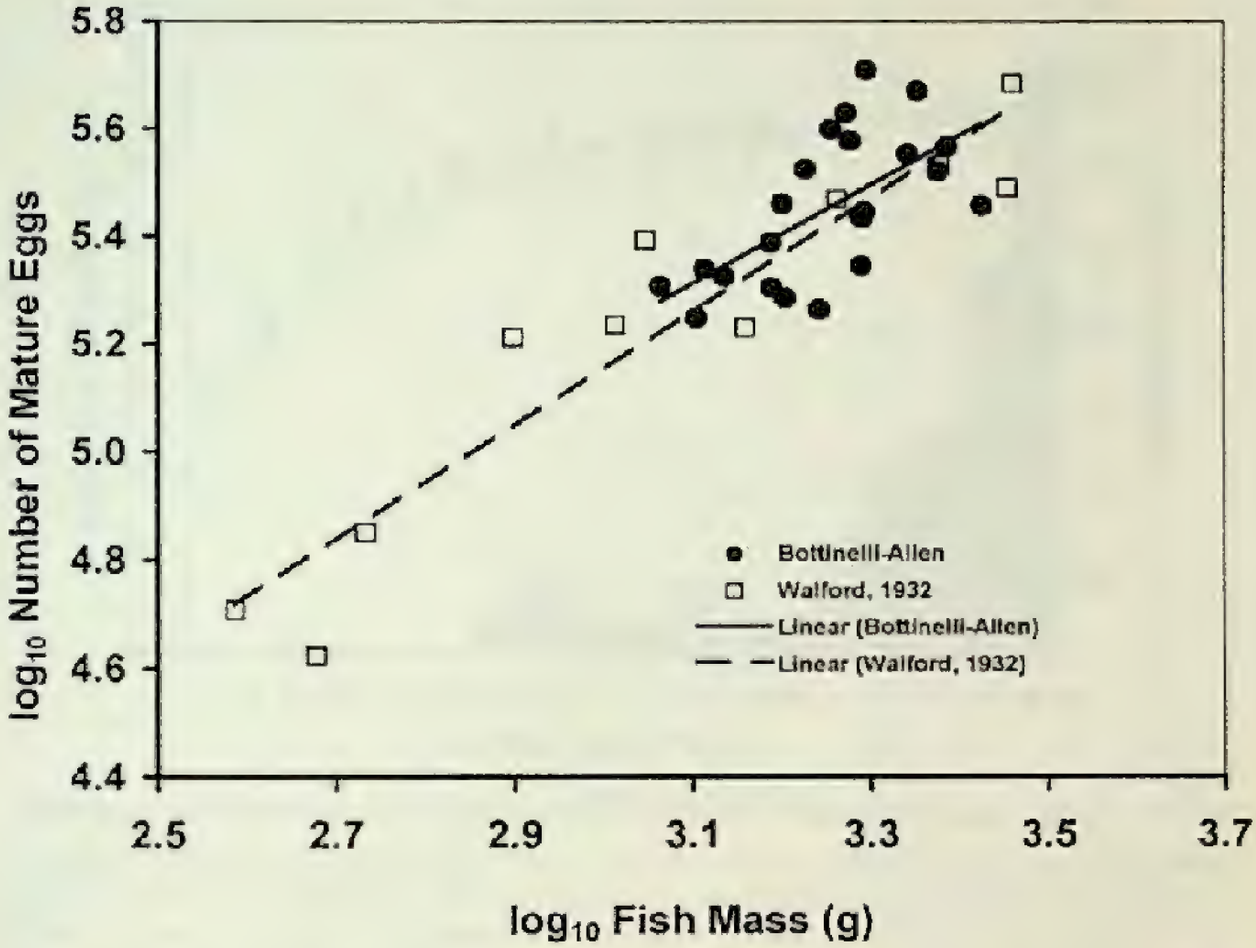


Figure 10. Linear regression line based on the log-transformed values for mass and the number of mature oocytes for the current study ($R^2 = 0.40$, df = 21, P = 0.001) and Walford (1932) ($R^2 = 0.89$, df = 9, $P < 0.001$).

first and second otolith readings was 74.2% (n = 609). A third examination resolved these discrepancies and increased the agreement to 98.5%.

The relationship between the $\log_{10}(x+1)$ of otolith width (mm) and the $\log_{10}(y+1)$ of age was described by the significant regression equation $y = 5.8572x - 3.6961$ ($R^2 = 0.86$, df = 590, $P < 0.001$). The significant regression equation: $y = 17.901x - 0.2386$, where x was the $\log_{10}(x+1)$ of otolith mass (g) and y was the $\log_{10}(y+1)$ of age, was a slightly better estimate of age ($R^2 = 0.88$, df = 528, $P < 0.001$). Therefore, these two morphometric characters can be used to predict age with a good degree of accuracy.

The mean standard lengths-at-age for all fish in the study regardless of sex (n = 596) were fit to the von Bertalanffy growth equation with the following results: $L_\infty = 774.3$ mm standard length, $k = .2506$, $t_0 = -2.745$ ($R^2 = 0.98$) (Fig. 12). Von Bertalanffy curves were also generated for fish of known sex (n = 405) (Fig. 13). The parameters for male fish (n = 196) were as follows: $L_\infty = 763.5$ mm standard length, $k = 0.2373$, $t_0 = -3.042$ ($R^2 = 0.96$). The parameters for female fish (n = 209) were slightly different: $L_\infty = 777.0$ mm standard length, $k = 0.2618$, $t_0 = -2.711$ ($R^2 = 0.97$).

Log-transformed values for both male and female fish were used to generate significant regression equations. Male fish were defined by: $y = 0.2753x + 2.5899$, where

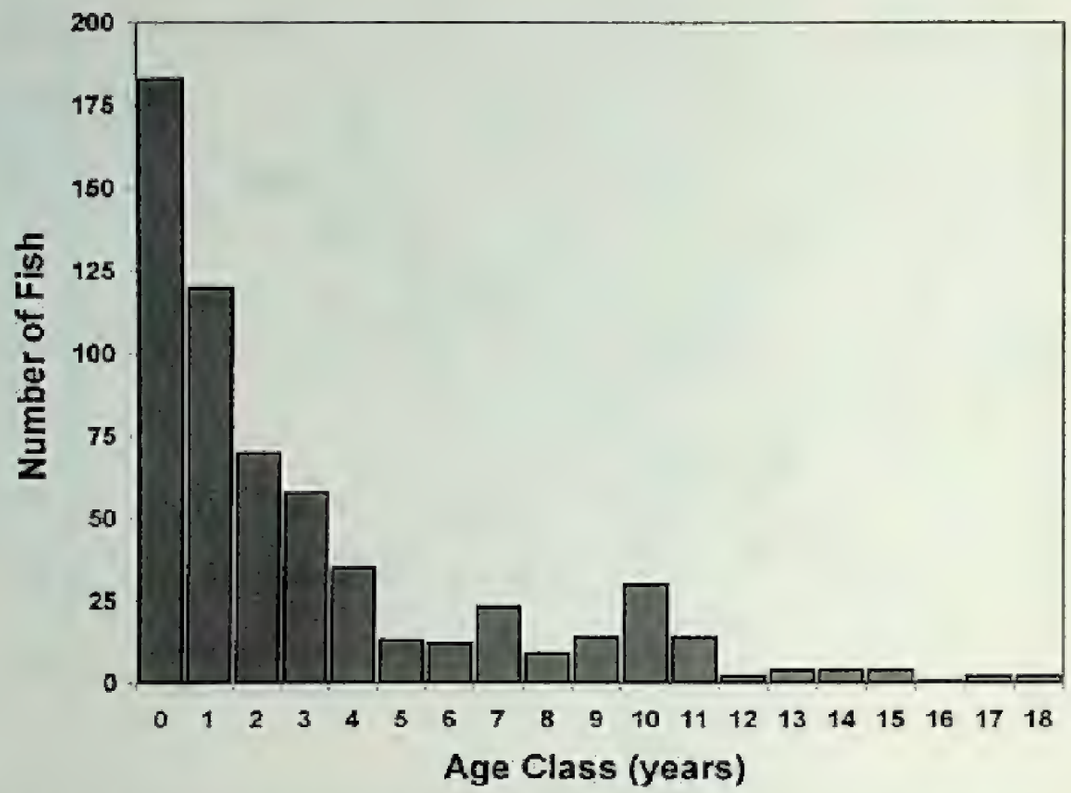


Figure 11. The age class frequency of all fish successfully aged by otolith examination (n=600).

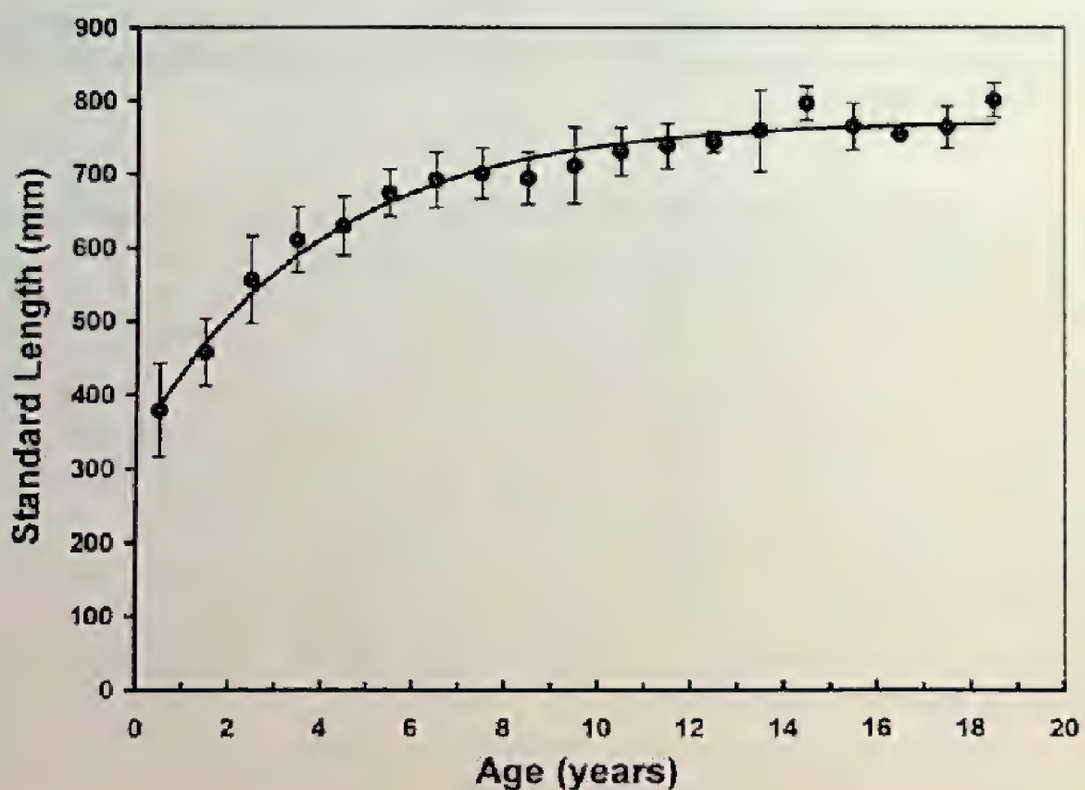


Figure 12. Growth curve for all fish (n = 596) based on the von Bertalanffy growth equation with the following parameters: $L_\infty = 774.3$, $k = .2506$, $t_0 = -2.745$ ($R^2 = 0.98$). Data points indicate mean length-at-age, and error bars represent one standard deviation.

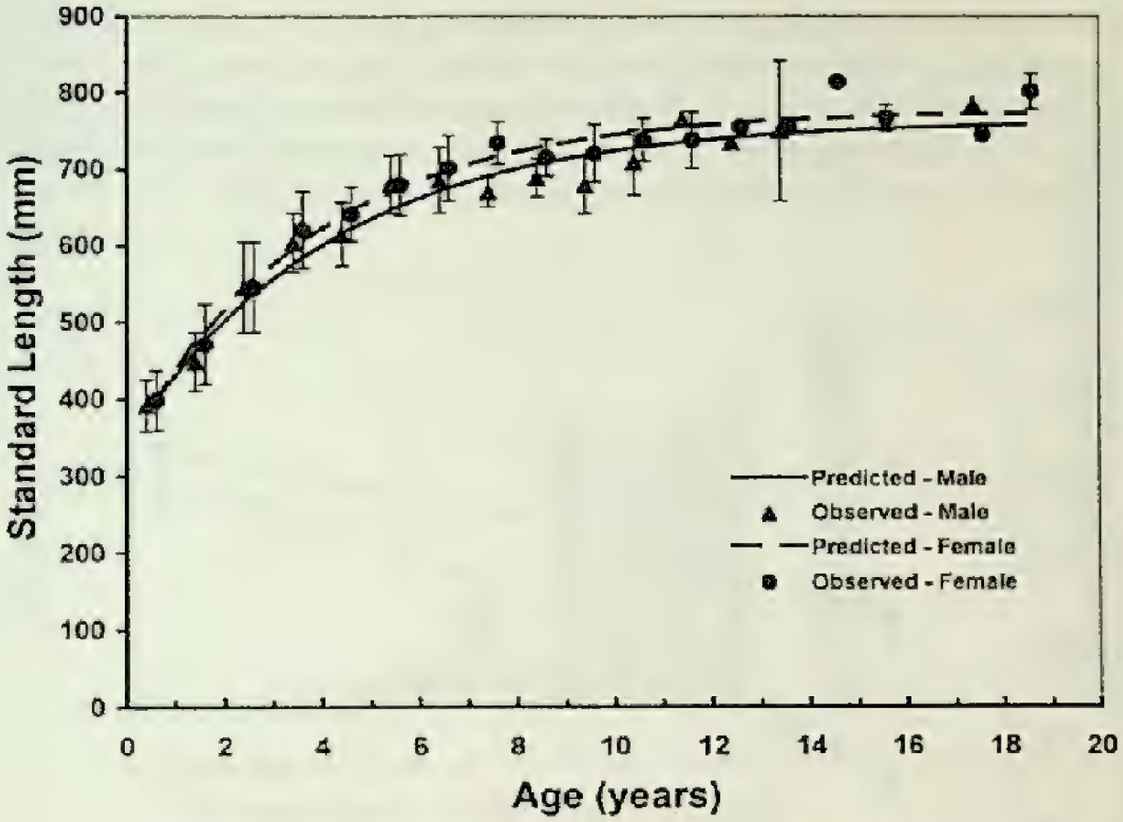


Figure 13. Growth curves for fish of known sex (n = 405) as calculated by the von Bertalanffy growth equation. The male curve is defined by the parameters L_∞ = 763.5, k = 0.237 and t_0 = -3.042 (n = 196, R^2 = 0.96). The female curve is based on L_∞ = 777.0, k = 0.2618 and t_0 = -2.711 (n = 209, R^2 = 0.97). Data points indicate mean length-at-age, and error bars represent one standard deviation.

y was the $\log_{10}(y+1)$ of standard length (mm) and x was the $\log_{10}(x+1)$ of age (years) (R^2 = 0.853, df = 194, P < 0.001). The significant female linear equation was: y = 0.2655x + 2.602 (R^2 = 0.86, df = 208, P < 0.001). The slopes of the two regression lines were homogenous (ANCOVA, F = 0.750, df = 1, 401, P = 0.387), but the magnitude of each line was significantly different (ANCOVA, F = 3.934, df = 1, 402, P = 0.048), indicating that female fish were longer at any given age.

In order to compare current growth rates of *Sphyraena argentea* to previous work it was necessary to generate von Bertalanffy parameters for Walford (1932). Values were calculated from published mean total length-at-age for age classes I through VI and were as follows: L_∞ = 894.3 mm total length, k = 0.3330, t_0 = -0.5150 (R^2 = 0.99). The data for the present study, after conversion to total length, produced the von Bertalanffy parameters: L_∞ = 902.1 mm total length, k = 0.2457, t_0 = -2.856 (R^2 = 0.98). Linear regression of mean length-at-age data for Walford (age classes I through IV) produced the significant equation y = 0.491x + 2.5524 (R^2 = 0.99, df = 2, P = 0.002) where y is the $\log_{10}(y)$ of total length (mm) and x is the $\log_{10}(x)$ of age (years). Log-transformed data for the current study (age classes I through IV) generated the significant regression equation: y = 0.2317x + 2.7354 (R^2 = 0.98, df = 2, P = 0.011). The slopes of these lines were significantly different (t = -10.36, df = 2, $P \leq 0.05$).

Von Bertalanffy parameters for Pinkas (1966) were published for age classes I-VIII and a growth curve was generated for age classes I-IV (Fig. 14). The significant regression equation $y = 0.4835x + 2.562$ fit the log-transformed data well ($R^2 = 0.99$, df = 2, $P = < 0.001$). The slope of the Pinkas (1966) regression line was also found to be significantly different from that of the current study ($t = -10.08$, df = 2, $P \leq 0.05$).

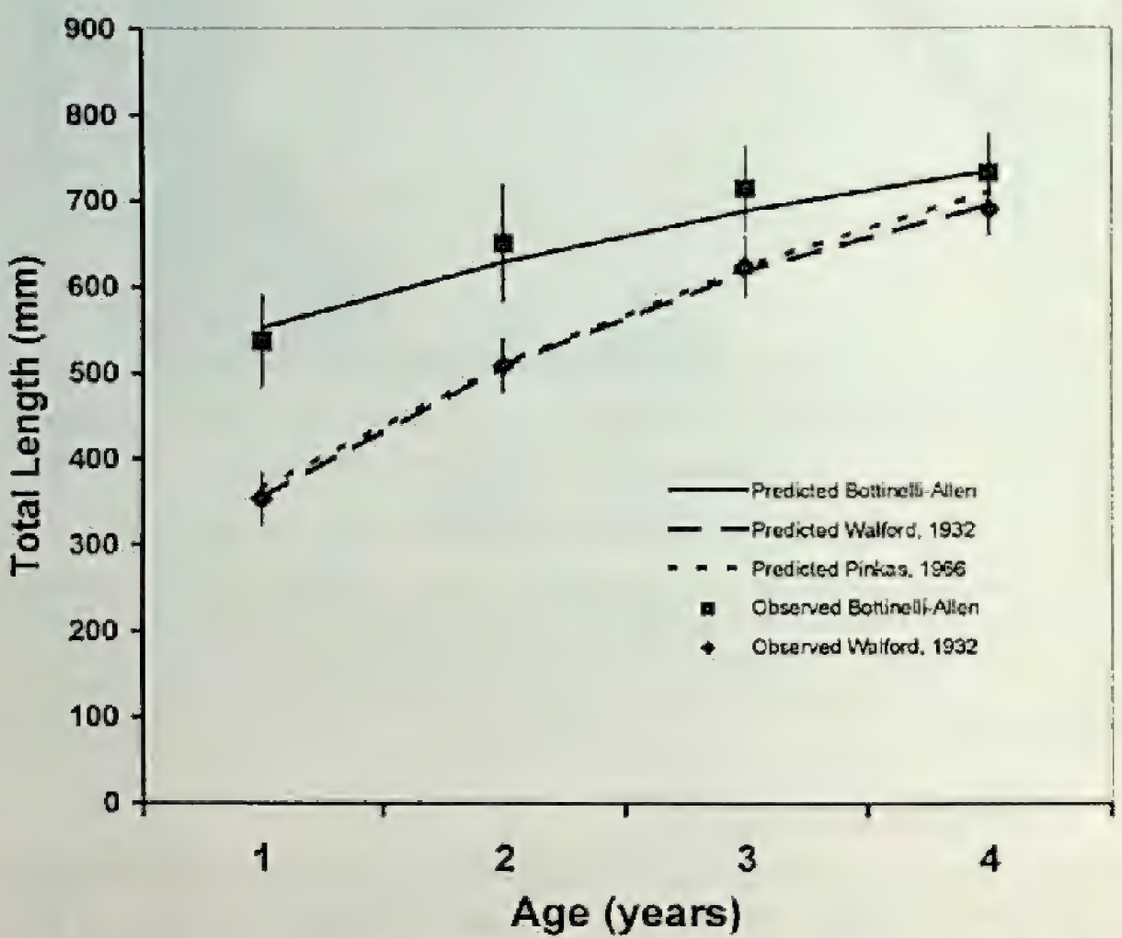


Figure 14. Growth curves for age classes I through IV from the current study, Walford (1932), and Pinkas (1966) as predicted by the von Bertalanffy equation. The Bottinelli-Allen curve is defined by the parameters $L_\infty = 902.1$, $k = 0.2457$, $t_0 = -2.856$ ($n = 596$, $R^2 = 0.98$). The Walford curve is based on $L_\infty = 894.3$, $k = 0.3330$, $t_0 = -0.5150$ ($R^2 = 0.99$). The Pinkas curve is based on the published parameters: $L_\infty = 1022.54$, $k = 0.2492$, $t_0 = -0.7689$. Data points indicate the actual mean length-at-age, and error bars correspond to standard deviation about that mean (Present Study and Walford only).

Age Determination from Scales

In the present study, 58 scales were successfully aged. The maximum age assigned by scale reading was 7 years. Percent agreement between first and second readings was 44.1% ($n = 59$). Only one instance of discordance could not be resolved with a third reading. Agreement after the third examination increased to 98.3%.

Disagreement between otolith-derived ages and ages assigned by scale analysis was common (Fig. 15). Agreement was high (less than 1.0 year difference) until the fifth

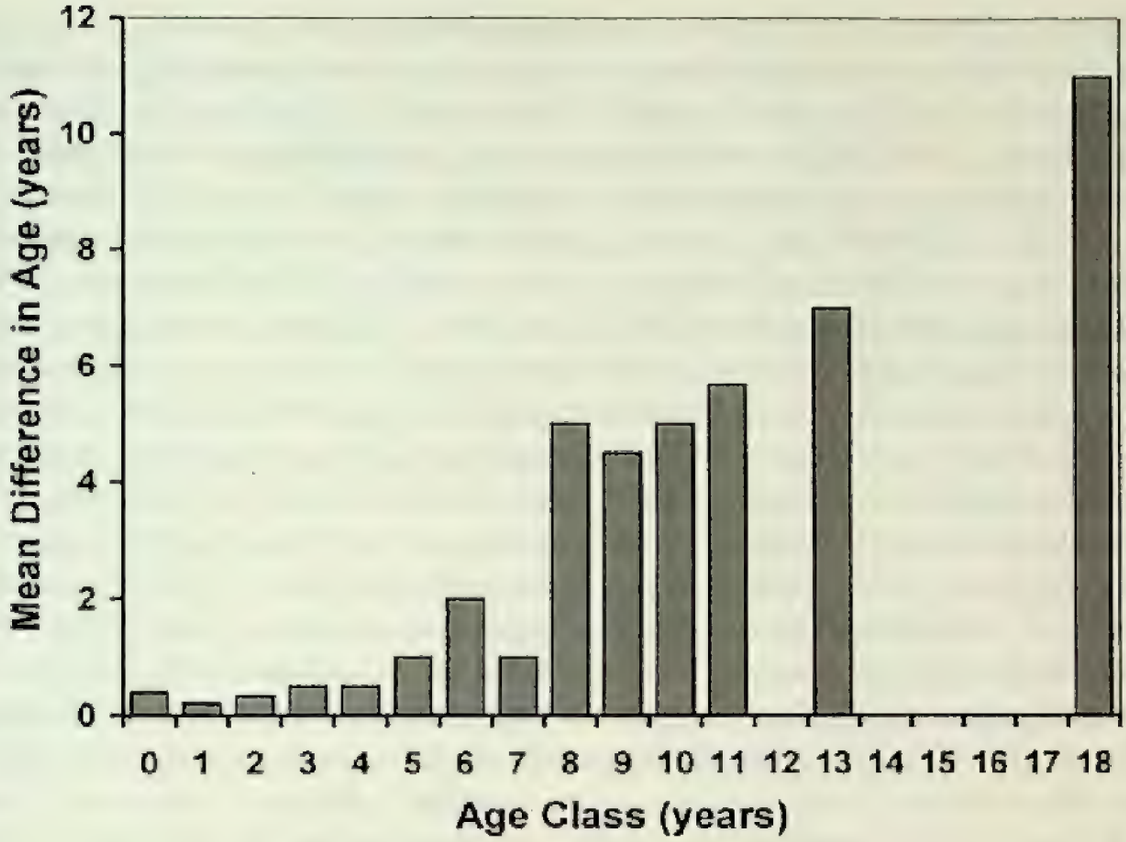


Figure 15. The mean difference in age estimates determined by scale and otolith analysis (n = 58).

year where the average discordance was one year. After age class VI, with the exception of VII, scale analysis underestimated the ages by at least 2 years. This error increased with age to a maximum error of 11 years for age class XVIII.

Linear regression of length-at-age data for age classes 0 through IV from the scale analysis resulted in the significant relationship $y = 0.3739x + 2.6155$ where y is the $\log_{10}(y + 1)$ of total length and x is the $\log_{10}(x + 1)$ of age ($R^2 = 0.74$, df = 37, $P < 0.001$). Corresponding data from otolith analysis produced the significant regression line $y = 0.3794x + 2.6167$ ($R^2 = 0.88$, df = 37, $P < 0.001$). The slopes of the two regression lines were homogenous (ANCOVA, $F = 0.017$, df = 1, 74, $P = 0.898$) and the magnitude of each line was also not significantly different (ANCOVA, $F = 0.105$, df = 1, 75, $P = 0.746$).

DISCUSSION

Length-Weight Relationship

Length-weight curves for fishes usually are a cubic function with the constant b » 3 (Walford 1932). Allometric growth of *Sphyraena argentea* (Fig. 5) was defined by a smaller exponent, which is characteristic of a slender species (deSylva 1963). This curve indicates a fairly slow weight gain as length increases for fish measuring less than about 400 mm standard length. Beyond this length, body mass increases at a much greater rate as the fish grows longer. This point roughly corresponds to the mean length

of age class I, showing very fast increase in length during the first year.

The reproductive behavior of *Sphyraena argentea* is undocumented, however, as a pelagic schooling fish they likely spawn in large groups (LGA, personal observation). Accordingly, they should lack the territoriality, complex mating behaviors, and propensity for sexual dimorphism associated with some substrate-oriented fishes. Male and female *S. argentea* do not differ in appearance except for anecdotal evidence that female fish have black anal fins while males have tan, grey, or yellowish anal fins (Love 1996). Considering these life history traits, female fish should weigh more than comparably sized male fish due to the size and weight of their gametes. In fact, the data presented herein suggest a contradictory trend. Males appear to be heavier than females of similar size (Fig. 6). Unfortunately, ANCOVA could not resolve differences in the magnitude of the regression lines because the slopes were not equal (Sokal and Rolf 1995).

When the difference in predicted mass between males and females is plotted against standard length, it is evident that the disparity in mass is small until the fish reach about 400-500 mm. After this point, males began to increase in mass at a greater rate than females with the disparity in mass reaching a maximum of more than 700 g at a standard length of 900 mm. Lengths at which female barracuda first develop mature gonads correspond well to the point where the growth curves of males and females start to diverge. The observed difference in mass is most likely due to the increased energy needs for ovarian development in female barracuda during the spawning season compounded by multiple spawning events per season.

A mature oocyte contains much more energy than a mature spermatozoon. In order to maximize the number of eggs produced per season, female barracuda must use more energy then males to create gametes, and thus will have less energy available for somatic growth (Wootton 1992). This alone could be enough to have a significant effect on the average weight of a female fish, but there is another factor: reproductive strategy. If *Sphyraena argentea* was a complete spawner, where all gametes were released once per season, then the allometric growth equation would be vastly different depending on when the fish were collected (before or after the single spawning event). However, the species is iteroparous, experiencing multiple spawning events per season. Therefore, the collections made for this study sampled female barracuda in various stages of gonadal development. A specimen collected soon after spawning essentially has had a large fraction of its mass deleted. The pooling fish of different reproductive states effectively underestimates the mean weight of female fish. Unfortunately, the gonads of male fish were not weighed or preserved making further evaluation of "gonad-free mass" impossible.

Compared to the Walford (1932) study, the allometric growth observed in the current study is significantly different (Fig. 7). Barracuda from the present study appear to weigh less than fish of comparable length examined by Walford (1932). The difference in mass becomes most noticeable in fish larger than 80 cm total length. The curve shown was plotted to a maximum length of 1 m, as the largest fish caught in this study had a total length of 952 cm. At this length, the difference in weight is approximately 250 grams. One obvious reason why the curves would differ could be measurement error. However, if that were the case, one would expect values to differ significantly along the entire

length of the curve and not only for larger fish. It is unlikely that only large fish were weighed incorrectly in either or both studies. A more likely explanation for the difference may be the source of the specimens studied. The length-weight curve for this study was based on fish caught during the months of April to October. Barracuda sampled during these months would have different masses depending on whether or not they had recently spawned. Furthermore, fish caught during April and October would have lighter gonads compared to a fish of similar length captured in midsummer (Fig. 8). The length-weight curve published by Walford (1932) was based on two groups of data: small fish that were collected from August 1 1927 to July 1 1928 and large fish sampled from the commercial fishery in June of 1928. Because smaller fish were sampled throughout the spawning season, they would contain gonads in various stages of development. However, the larger, market sized fish were only collected in June during the peak spawning season. Thus, the larger specimens used in the length-weight calculations would likely include more individuals with massive gonads. In fact, the point at which the curves start to diverge is approximately the legal size limit of ~720 mm total length. The observed difference in the length-weight curves for these two studies, while statistically significant, is probably not biologically relevant and likely caused by the differing sources of specimens used by Walford (1932) and the current study.

Spawning Season and Batch Fecundity Estimate

Spawning season for *Sphyraena argentea* has been previously studied and determined to last from April to September, with peak season from mid-May to mid-July. The present study used gonosomatic indices (GSI) to confirm the spawning season of *S. argentea* (Fig. 8). High monthly standard deviation in the summer and low deviation in the winter is indicative of multiple spawning events. Months with higher standard deviations would indicate a mix of fish with different GSI. Those individuals with heavy, hydrated gonads would have very high GSI relative to those who had just completed a spawning event, thus increasing the standard deviation. The month of June has the highest standard deviation, which indicates a mix fish of different reproductive states , and also is ranked second in terms of average GSI. During April, the spawning season is just starting, as demonstrated by the low GSI and low standard deviation. In October, the situation is similar as the spawning season is ending, and most fish would be expected to have already released all of their mature oocytes. This data suggests that spawning season peaks in June and agrees with previous studies in this regard.

Batch fecundity was estimated by counting mature oocytes. The mass of the fish proved to be the best indicator of the number of mature oocytes in the gonad (Fig. 9). This is to be expected as fecundity in fishes is often correlated best with the mass of the fish (Nikolskii 1969; DeMartini and Fountain 1981). A change in batch fecundity in *Sphyraena argentea* might be cause for concern as fecundity is related to environmental conditions such as food supply (Nikolskii 1969). Current batch fecundity, as a factor of mass, was not significantly different from previous estimates (Fig. 10). Batch fecundity was not found to be significantly related to total length by current data.

Additionally, this factor could not be compared to Walford (1932) using ANCOVA because the slopes of the two regression lines were not homogenous. The elevations of the two regression lines based on the other factor of interest, age, could also not be analyzed using ANCOVA due to significantly different regression slopes. Walford (1932) contained the length, weight, age, and batch fecundity data for 11 fish–4 of which were age 6 or 7. The error in age estimation of older fish by Walford (1932) is probably the cause of the disparity between the regression slopes.

Age Determination – Otoliths

Disagreement between the first and second otolith readings did occur, but was not problematic because most barracuda (98.5%) were assigned reliable ages after a third reading. Initial underestimation of age by 1 year was the most common cause of discordance between first and second examinations (n = 122) and occurred most often in age I fish. Failure to count the first annulus occurred most often in 1-year-olds because this annulus is sometimes difficult to discern in the absence of other annuli. The first annulus can also be fainter than subsequent annuli (Barbieri et al. 1993) leading to underestimation of older fish. Discrepancies of 2 years were very rare, occurring in only seven otoliths. There were no cases where the second reading was in error by more than 2 years.

Edge analysis, specifically noting whether an opaque or translucent band is present at the otolith edge, is a widely accepted method of validating that the bands observed are indeed annuli. This was the method originally planned for use in the present study, however, complications arose as sections were examined. During preparation for sectioning, as mentioned above, otoliths were glued to blocks of wood using cyanoacrylate glue. While this method has been employed before successfully with many species, some considerations are warranted if future work with *Sphyraena argentea* is attempted. Otoliths of *S. argentea* are normally curved, possessing a convex and relatively flat to slightly concave surface and rest naturally with the convex surface facing the ground. It was in this manner that sagitta were permanently attached to wood blocks before sectioning. This orientation made edge analysis impossible as the edge in question was confounded by the clear cyanoacrylate (Fig. 3). Therefore, this method of validation was deemed unreliable and subsequently abandoned.

Otolith morphometrics and their relation to assigned ages were used instead to confirm the annual nature of opaque bands in *Sphyraena argentea*. Both otolith width ($R^2=0.86, P<0.001$) and otolith length ($R^2=0.88, P<0.001$) were closely correlated with age. These analyses, while they do not definitively validate age, are a good indication that the opaque bands seen in *S. argentea* sagitta are in fact annuli, and can be used reliably as aging structures. Additionally, the temperate range of the species exposes them to seasonality in water temperatures, which supports the probability of annual formation of opaque bands during warmer months. Future attempts at aging should mount otoliths with the concave surface facing up, or employ a different method of mounting or embedding.

The growth curve for all fish generated from the von Bertalanffy equation (Fig. 12)

indicated very fast growth during the first year (mean Age O = 379 mm). Rapid growth of young-of-the-year is characteristic of most fishes (Wooten 1992) and has been observed in other *Sphyraenidae* (DeSylva 1963). The slackening in growth observed as fish age may be due to a host of factors such as decreased ration size relative to body mass as the fish grows, and the energetic needs of reproduction (Wooten 1992).

Similar growth patterns were observed when male and female fish were fit separately to von Bertalanffy curves (Fig. 13). Walford (1932) found no significant length difference between the sexes "until the 4th year, when females appear to be 3 per cent larger". However, this study found a difference in length at all ages that peaks at about 3.5% (age classes III-V). This is not an unknown phenomenon to fishery science and has been observed in many species (Beckman et al. 1988; Lea et al. 1999) but curiously not in the great barracuda, *Sphyraena barracuda* (DeSylva 1963).

Age Determination – Scales

The examination of *Sphyraena argentea* scale annuli provided an unreliable estimate of age when compared to otolith annuli. Fish from age classes 0 to XVIII (as determined by otolith examination) were included in the scale study but the maximum number of annuli counted in any scale was seven. This agrees with Walford (1932) when he writes, "spaces between the first five annuli were sufficiently wide for reasonably consistent and accurate counting. Those beyond six became too narrow, and the year marks too crowded, to justify any attempt at trustworthy age determination beyond six years". In fact, the problem with scale analysis in *S. argentea* is not simply that fish over age 6 cannot be aged reliably (Fig. 15). It is that scales from fish over age 6 will be recorded as 6 year olds, thus underestimating the age of all older fish.

Sphyraena argentea scale annuli were much more difficult to see and interpret when compared to the otolith annuli of the same fish. More time had to be spent on each sample and the final reading was based on a high degree of subjective interpretation of exactly what was an annulus. Also, many of the scales examined were torn or damaged, possibly from extraction and storage. Scales from the same fish would sometimes appear to be a different age, indicating possible regeneration of lost scales. It is our opinion that analysis of *S. argentea* scales is subject to a very high degree of error and should not be used when sagittal otoliths are available for study.

Comparison of Growth Rates to Previous Studies

The growth curves generated for the historical data presented in Walford (1932) and Pinkas (1966) differ markedly from the results of the current study (Fig. 14). The growth curves for Walford (1932) and Pinkas (1966) were based on scale analysis while the current study used sagittal otoliths. However, the ages assigned by these two methods did not differ significantly from one another for age classes 0 to IV, which justifies the comparison of these curves. Clearly, the growth rate of fish caught for this study is much higher during the first few years than the rate seen in both previous studies, which appear to be almost identical. The curves do start to converge indicating that the higher

growth rate is confined to the first 2 or 3 years. This disparity in growth can be attributed to many factors, the first of which is the methodology of each study.

The fish examined for the 1932 study by Walford were collected from two main sources: commercial fishing boats and what Walford has called "special collections" of younger fish. The commercial catch, which was sampled at various landings throughout southern California, did not include fish smaller than the legal size limit of 3 lb (about 720 mm total length). In order to collect younger fish, two commercial fishermen were given collecting permits and instructed to target smaller barracuda. Compounding the problem is the fact that these fishermen were paid by the piece for very small barracuda and by the pound for larger barracuda. Walford states, "Since the second year fish were nearly all part of the special collections of young fish, the skewed nature of the II year class curve is doubtless due to a selection by the fishermen against the larger sizes". This lead to a situation where there were not many large 2-year olds caught, which would artificially drive down the mean length of the second year class. The third year class is similarly underestimated: the commercial fleet will not catch many 3-year olds because they are too small to legally keep, and the special collections will underestimate the mean length by targeting smaller fish.

Pinkas (1966) also examined samples obtained from commercial fishermen. Smaller fish were obtained from commercial passenger fishing vessel patrons who were permitted by law to keep two undersized (less than 711 mm total length) barracuda per day. Sampling in this manner would reduce the number of young fish sampled, but not necessarily reduce the mean length of each age class. Why then are the curves from these two studies nearly identical and, more importantly, why do they differ so dramatically from the current study?

A likely explanation for the apparent increase in growth rate of *Sphyraena argentea* seen in the current study, is the change in sea surface temperature associated with the Pacific Decadal Oscillation (PDO). The PDO is a large-scale oceanographic process characterized by alternating regimes of cool and warm water. Average temperature associated with these regimes only differs by about 1 or 2 degrees C, but these changes affect a large area of the Pacific for a long period of time (Mantua et al. 1997). The regime switch occurs roughly every 25 years and has been shown to affect biota from zooplankton to seabirds, but is best known for its correlation with the rise and fall of northern anchovy, *Engraulis mordax*, and Pacific sardine, *Sardinops sagax*, populations (Chavez et al. 2003; Horn and Stephens 2005). The California barracuda feeds on both sardines and anchovies and thus should be little troubled by a regime change from the perspective of diet. However, sea temperature, which drives the biological regime shifts, can have a large effect on growth rate.

Fish growth is determined by many environmental factors, one of the most important is temperature (Nikolskii 1969; Helfman et al. 1997; Moyle and Cech 2000). Water temperature is positively correlated with an increase in the rate of gastric evacuation, which allows a corresponding increase in the rate of food consumption. Fish metabolism also rises with temperature, due to an increase in the rate of chemical reactions at the cellular level (Wootten 1992). This increase in metabolism is fueled by the increased feeding rate. Energy available to somatic growth should continue to

increase if food is abundant. However, if food is limited, an increase in temperature will cause a decrease in growth rate. Additionally, an increase in temperature past a species-specific optimal temperature, regardless of food supply, will be detrimental (Wootten 1992).

Walford (1932) published mean length-at-age data that were based on fish collected in 1928. The von Bertalanffy parameters published in Pinkas (1966) are based on data collected during 1960. Of interest is the fact that these two studies used barracuda caught near the end of the cool regime ending around 1925 (Walford) and during the middle of the 1950-1975 cool period (Pinkas) (Fig. 16). Fish collected for study by Pinkas (1966) would have grown up during a cool PDO regime. Specimens used by Walford (1932) would have primarily grown up during the latter part of a cool regime or during the transition into a warm regime. The last warm regime is thought to have ended sometime in the late 1990's (Chavez et al. 2003) with 1999 gaining consensus as the year of the switch (Horn and Stephens 2006.). Most of the specimens for the current study were collected in 2000-2002 and would have spent most of their lives in the end of a warm regime.

Another oceanographic phenomenon, the El Nino Southern Oscillation (ENSO), may also have played a part in the increased growth rates observed in the current study. In 1997-98 an exceptionally warm ENSO event occurred which kept sea surface temperature elevated during the waning years of the latest warm PDO regime. These two oceanographic warming events, occurring at the same time, could have had a positive influence on the growth rate of fishes during the first few years of life. Therefore, we propose that the difference in growth rates observed may be due to the fact that the barracuda collected for previous studies grew up in colder water than those collected for the current analysis.

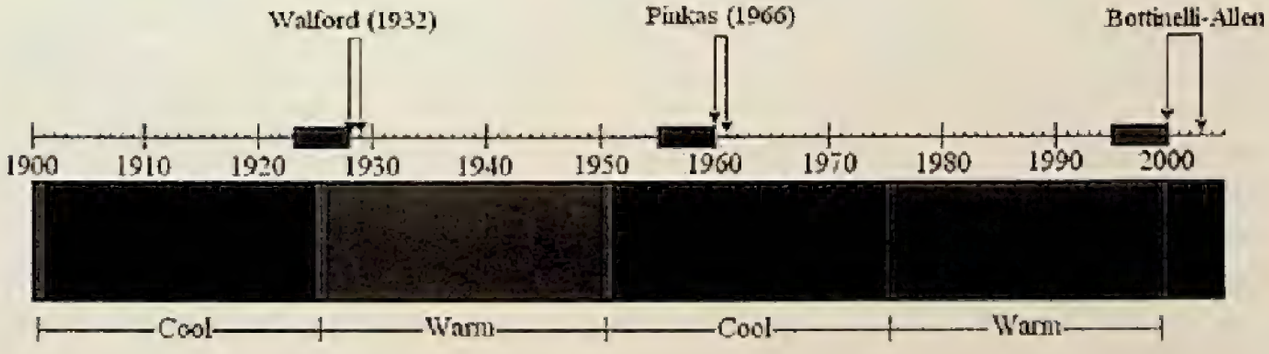


Figure 16. Timeline showing the cool and warm water regimes associated with the Pacific Decadal Oscillation (Chavez et al. 2003). Arrows indicate the time periods when *Sphyraena argentea* were being collected for Walford (1932), Pinkas (1966) and the current study. Shaded blocks on the timeline itself mark a period of five years before collections are made, which represent age classes 0-IV. Smaller shaded areas at the border of warm and cool periods represent the year of transition between PDO regimes.

Conclusion and Implications for Management

Sphyraena argentea is an species that is routinely targeted by the recreational fishery in southern California for sport and consumption. Commercial fisheries do not heavily exploit it at the present time. However, this situation could change as stocks of local species more popular as food continue to decline and the costs of importing fishes from abroad rise. Barracuda are an edible, fast growing, easily captured species that could become more popular as landings of other species decrease due to overfishing and associated regulatory actions. This is evident in the recent explosion in the landings of another fast growing, edible animal, the market squid, *Loligo opalescens*. Landings (recreational and commercial) of *L. opalescens* have dramatically increased since the late 1980's, primarily due to increased demand from overseas markets.New fisheries regulations have since been drafted to deal with the increased pressure on the squid stocks (CDFG, 2005). If future circumstances necessitate the drafting of similar plans for the California barracuda, this study will provide important contemporary estimates of growth and batch fecundity that will be useful in the short and long-term management of the resource.

The reevaluation of the age structure of the species with sagittal otoliths has shown them to be a much longer-lived species than previously thought which is positive news for the future of the population. Longevity is an important trait to consider. The capture of an individual that has just reached maturity does not simply remove one barracuda from the population; it prevents the release of hundreds of thousands, perhaps millions, of viable eggs per year for the life of the fish. The stock management of a fish known to live at least 18 years should be different from that of a species thought to reach a maximum age of 11. Indeed, a longer life means more spawning opportunities, which may allow for a higher maximum sustainable yield. However, if regulations allow for the harvest of young animals that have had few opportunities to spawn, limited benefit will be derived. The current minimum size limit of 28 inches TL (about 610 mm SL) seems to protect barracuda from harvest until about the third or fourth year of life. Barracuda become sexually mature early in life at approximately 350 to 475 mm SL, which is during their first or second year (Walford 1932). Therefore, the current size limit only allows barracuda two or three years of reproductive opportunity before they can be legally captured. If fishing pressure increases and revised regulations are necessary, a slight raise in the minimum size limit would provide a few additional years of reproductive output. It should also be noted that an increase in the legal length limit would protect much heavier fish, as mass is a cubic function of length. This is notable because batch fecundity is significantly correlated to mass. Therefore, the protection of larger fish based on their high reproductive potential should be addressed. The possible use of "slot-limits" should be considered where, in addition to a minimum size limit, fish of "trophy size" are also protected.

Harvest regulations must not be static. Understanding how fish populations are affected by periodic climatic changes, such as the Pacific Decadal Oscillation (PDO), is essential to their proper management. This study demonstrated a possible increase in the growth rate of California barracuda during the most recent warm period of the

PDO. Fishing regulations based on data from warm periods may not be as effective during cooler periods and may work to hinder the sustainable nature of some fisheries. For example, minimum size and bag limits enacted during periods of cool water and slower growth may allow a larger proportion of younger fish to be harvested during warm PDO regimes. If more young fish (relative to fish of similar length in a cool period) are taken from the population, the reproductive output of the stock may suffer as individuals are alive for fewer spawning seasons. Oceanographic processes like the PDO, and their direct and indirect effects on commercially important fishes, must be better understood in order to forecast the viability of stocks and draft new harvest regulations. Proper stewardship of our ocean resources demands future investigation.

ACKNOWLEDGMENTS

We want to thank P. Edmunds, M. Franklin, D. Pondella for their tireless assistance during the course of this study. The entire Fish Lab at CSUN, past and present, deserve thanks but a few in particular went above and beyond. B. Erisman, M. Adreani, E. Forsman, J. Cobb, J. Froeschke , E. Miller, M. Salomon, and J. Lindsay are greatly appreciated for their help with field collections. We also acknowledge the many folks from the Vantuna Research Group, and the crew of the *R/V YELLOWFIN* and *R/V VANTUNA* for their help in the field. Many of the specimens used in this study were collected in conjunction with the gill net sampling program targeting juvenile white seabass funded by the California Department of Fish and Game through the Ocean Resource Enhancement and Hatchery Program (OREHP).

LITERATURE CITED

Allen, L.G. 1985. A habitat analysis of the nearshore marine fishes from Southern California. Bulletin Southern California Academy Sciences 84(3):133-155.

Allen, L.G., T.E. Hovey, M.S. Love, and J.T.W. Smith. 1995. The life history of the spotted sand bass (*Paralabrax maculatofasciatus*) within the Southern California Bight. California Department Fish and Game CalCOFI Reports 36:193-203.

Allen, L.G., A.M. Findlay, and C.M. Phalen. 2002. The fish assemblages of San Diego Bay in the five-year period of July 1994 to April 1999. Bulletin Southern California Academy Sciences, 101(2): 49-85.

Ally, J.R.R. and K. Miller. 1992. California barracuda. *In* W.S. Leet, C.M. Dewees and C.W. Haugen (eds.), California's living marine resources and their utilization, p. 157-160. California Sea Grant Extension Program, Dept. of Wildlife and Fisheries Biology, University of California, Davis. California Sea Grant Extension Publication UCSGEP-92-12.

Barbieri, L.R., M.E. Chittenden Jr., and C.M. Jones. 1994. Age, growth, and mortality of Atlantic croaker, *Micropogonias undulates*, in the Chesapeake Bay region, with a discussion of apparent geographic changes in population dynamics. United States Fishery Bulletin 92:1-12.

Beckman, D.W., C.A. Wilson, and A.L. Stanley. 1988. Age and growth of red drum, *Sciaenops ocellatus*, from offshore waters of the northern Gulf of Mexico. United States Fishery Bulletin 87:17-28.

Beckman, D.W. and C.A. Wilson. 1995. Seasonal timing of opaque zone formation in fish

otoliths. *In* D.H. Secor, J.M. Dean, and S.E. Campana (eds.) Recent Developments in Fish Otolith Research, p. 27-43.

Cailliet, G.M., M.S. Love, and A.W. Ebeling. 1986. Fishes: a field and laboratory manual on their structure, identification, and natural history. Wadsworth Publishing Co., Belmont, CA. 194 pp.

Chavez, F.P., J. Ryan, S.E. Lluch-Cota, M. Ñiquen C. 2003. From anchovies to sardines and back: multidecadal change in the Pacific Ocean. Science 299:217-221

CDFG (California Department of Fish and Game) 2005. Market Squid Fishery Management Management Plan 2005. Annual Review. Calif. Dept. Fish Game, Marine Region. 14 pp.

Craig, M.T., D.J. Pondella, II and J.C. Hafner. 1999. Age and growth in two eastern Pacific groupers (Serranidae: Epinephelinae). Bulletin of Marine Science 65(3):807-814.

DeMartini, E.E. and R.K. Fountain. 1981. Ovarian cycling frequency and batch fecundity i n the queenfish, *Seriphus politus*: attributes representative of serial spawning fishes. United States Fishery Bulletin 79(3):547-560.

DeSylva, D.P. 1963. Systematics and life history of the great barracuda. Univ. Miami Studies in Tropical Oceanography 1:1-179

Fitch, J.E. and R.J. Lavenburg. 1971. Marine food and game fishes of California. University of California Press, Berkeley. 179p.

Follett, W.I. 1976. Fish remains from an archaeological site at Rancho Carrillo on the Silver Strand, San Diego County, California. Bulletin Southern California Academy Sciences 75(2):131-137.

Helfman, G.S., B.B. Collette, D.E. Facey. 1997. The Diversity of Fishes. Blackwell Science, Malden, MA. 528p.

Horn, M.H. and J.S. Stephens, Jr. 2006. Climate change and overexploitation *In* L.G. Allen, D.J. Pondella II, and M.H. Horn (eds.), The Ecology of Marine Fishes: California and Adjacent Waters. University of California Press, Berkeley, CA. p. 621-635.

Hunter, J.R. and S.R. Goldberg 1980. Spawning incidence and batch fecundity in northern - anchovy, *Engraulis mordax*. United States Fishery Bulletin 77(3):641-652.

Lai, H., V.F. Gallucci, and D.R. Gunderson.1996. Age determination in fisheries: methods and applications to stock assessment. *In* V.F. Gallucci, S.B. Saila, D.J. Gustafson, and B.J. Rothschild (eds.), Stock Assessment: Quantitative Methods and Applications for Small-Scale Fisheries. Lewis Publishers, New York. p. 82- 178.

Lea, R.N., R.D. McAllister and D.A. VenTresca. 1999. Biological aspects of nearshore rockfishes of the Genus *Sebastes* from central California. California Department Fish Game, Fishery Bulletin, 177. 109p.

Love, M. 1996. Probably more then you want to know about the fishes of the Pacific coast. Really Big Press. Santa Barbara, CA. 381p.

Lowerre-Barbieri, S.K., M.E. Chittenden Jr., and C.M.Jones. 1994. A comparison of validated otolith method to age weakfish, *Cynoscion regalis*, with the traditional scale method. United States Fishery Bulletin 92:555-568.

Mantua, N.J., Hare, S.R., Wallace, J.M, and R.C. Francis. 1997. A Pacific decadal climate oscillation with impacts on salmon production. Bulletin American Meteorological Society 78(6):1069-1079.

Moser, H.G., R.L. Charter, P.E. Smith, D.A. Ambrose, W. Watson, S.R. Charter, and E.M. Sandknop. 2001. Distributional atlas of fish larvae and eggs in the Southern California Bight region: 1951-1998. California Department Fish Game CalCOFI Atlas 34:49.

Moser, H.G., R.L. Charter, P.E. Smith, D.A. Ambrose, W. Watson, S.R. Charter, and E.M. Sandknop. 2002. Distributional atlas of fish larvae and eggs in the Southern California Bight region: 1977-2000. California Department Fish Game CalCOFI Atlas 35:23.

Moyle, P.B. and J.J. Cech, Jr. 2000. Fishes: An Introduction to Ichthyology. 4th Edition. Prentice Hall, Upper Saddle River, NJ. 612p

Nikolskii, G.V. 1969. Theory of fish population dynamics as the biological background for rational exploitation and management of fishery resources. Oliver & Boyd, Edinburgh. 323p.

Pinkas, L. 1960. California barracuda (*Sphyraena argentea*). *In* California Ocean Fisheries Resources to the Year 1960. California Department Fish Game. pp 22-24

Pinkas, L. 1966. A management study of the California barracuda, *Sphyraena argentea* Girard. California Department Fish Game, Fish Bulletin. 134. 58 pp.

Roedel, P.M. 1948. Common marine fishes of California. California Department Fish Game, Fishery Bulletin 68:55.

Schultze, D.L. 1983. California barracuda life history, fisheries, and management. California Department Fish Game CalCOFI Rep. 24:88-96.

Secor, D.H., T.M. Trice, and H.T. Hornick. 1995. Validation of otolith-based ageing and a comparison of otolith and scale-based ageing in mark-recaptured Chesapeake Bay striped bass, *Morone saxatilis*. United States Fishery Bulletin 93:186-190.

Secor, D.H., Dean, J.M., and Campana, S.E. 1995a. Introduction *In* D.H. Secor, J.M. Dean, and S.E. Campana (eds.) Recent Developments in Fish Otolith Research, p. xxv-xxvii.

Sokal, R.R. and F.J. Rohlf. 1995. Biometry. 3rd Edition. W.H. Freeman and Co., NY. 887p.

Walford, L. 1932. The California barracuda (*Sphyraena argentea*). California Department Fish Game, Fishery Bulletin 37:1-72.

Weatherley, A.H. 1972. Growth and Ecology of Fish Populations. Academic Press, New York. 293pp.

Wootten, R.J. 1992. Ecology of Teleost Fishes. Chapman and Hall, London. 404p.

Wright, P.J. 1991. The influence of metabolic rate on otolith increment width in Atlantic salmon parr, *Salmo salar* L. Journal Fish Biology 38:929-933.

Received: 26 July 2006
Accepted: 26 November 2006

California Fish and Game 93(4):200-213 2007

COLD TEMPERATURE-INDUCED OSMOREGULATORY FAILURE: THE PHYSIOLOGICAL BASIS FOR TILAPIA WINTER MORTALITY IN THE SALTON SEA?

BRIAN A. SARDELLA* AND COLIN J. BRAUNER
Department of Zoology
University of British Columbia
Vancouver BC, V6T 1Z4 Canada
*Current email: basardella@ucdavis.edu

ABSTRACT

The Salton Sea is a large saline lake in southeastern California that has been plagued by large-scale mortality among the fishes that once comprised a robust recreational fishery. In addition to very high salinity (43-47 g/l), other stressors such as large fluctuations in temperature exist that when combined with salinity can result in a severe physiological challenge to resident fishes. Fish kills during winter months are predominantly comprised of 'California' Mozambique tilapia, *Oreochromis mossambicus* x *O. urolepis hornorum*, so we investigated the effects of temperature and salinity interaction as a cause of the mortality. Tilapia acclimated to 43 g/l were transferred from 25 °C to 15 or 35 °C in order to assess the effect on osmoregulatory ability. There were significant increases in plasma osmolality and [Na^+] 24 h after tilapia were transferred to 15°C that recovered to pre-transfer values by 120 h. In order to demonstrate the additive effects of temperature and salinity, we transferred tilapia from 35 g/l salinity at 25 °C to 43 g/l salinity at 15, 25, and 35 °C. The combined temperature and salinity challenge significantly affected plasma osmolality and branchial Na^+, K^+-ATPase (NKA) activity, with fish in the 15 °C group experiencing mortality after 12 h. Branchial NKA activity was significantly increased 3 h following transfer, but was reduced by 6 h in the 15 and 35 °C groups. The ability of this species to effectively osmoregulate as well as tolerate salinity transfer was impaired following a reduction in ambient temperature. Based on these experimental findings, we concluded that cold-induced tilapia mortality during winter months at the Salton Sea is due to osmoregulatory failure.

INTRODUCTION

The Salton Sea is a large (980 km^2) inland lake that was created in the early 20th century when water from the Colorado River flooded the Imperial Valley, forming a large sea in an arid desert. Due to inflow of nutrient- and salt-rich water, and a very high rate of evaporation, the salinity of the Salton Sea has increased from near freshwater at the time of its formation to 44 g/l presently, and continues to increase at a rate of 0.3 g/l/year (Watts et al. 2001). Despite its physiologically-challenging environment, the Salton Sea has supported a substantial fish population for decades. Over 30 species

have been introduced into the Sea during the last 80 years; however, only orangemouth corvina, *Cynoscion xanthulus*, bairdiella, *Bairdiella icista*, and sargo, *Anisotremus davidsoni*, have flourished since the mid-1950s (Walker et al. 1961). The dominant fish species since the mid-1960s has been a Mozambique tilapia hybrid, *Oreochromis mossambicus* x *O. urolepis hornorum*, which is also referred to as the California Mozambique tilapia (Costa-Pierce and Doyle 1997). Together these four species comprise a large portion of the total Salton Sea fishery, and have all been shown to tolerate high salinities in laboratory studies (Hanson 1970; Sardella et al. 2004b). However, there are other stressors playing deleterious synergistic roles in the Salton Sea, including a large seasonal temperature range (12-35°C), high [SH^-] (up to 5 mg/l), high [NH_3] (1.2 mg/l), toxic metalloids such as selenium and arsenic, low dissolved oxygen levels with occasional anoxia, and various disease outbreaks (Gonzalez et al. 1998; Watts et al. 2001; Riedel et al. 2002). The continual salinity increase of the Salton Sea is a major concern for conservation agencies such as the Salton Sea Authority. Fish kills occur there throughout the year, with a peak in mortality rate typically during the hot summer months. Unlike corvina, croaker, and sargo, tilapia are also subject to a high rate of mortality during the winter (Hurlbert et al. 2007), which may result from a low tolerance for cold temperatures (Al Amoudi et al. 1996, Sardella et al. 2004a). While summer mortality is typically attributed to low oxygen availability and high [SH^-] (Watts et al. 2001; Hurlbert et al. 2007), the etiology of the winter tilapia kills has yet to be fully elucidated.

At 25 °C, California Mozambique tilapia were able to tolerate salinities as high as 95 g/l, with sub-lethal disturbances observed in salinities greater than 65 g/l (Sardella et al. 2004b). This impressive salinity tolerance has also been documented in pure Mozambique tilapia (Stickney 1986), as has this species inability to tolerate cold ambient temperatures (Al Amoudi et al. 1996). The Salton Sea has a wide annual temperature range (12 - 35 °C), and regularly windy conditions can result in a complete turnover of the lake, making it difficult for fish to avoid temperature extremes (Watts et al. 2001). In the few studies conducted to date, changes in temperature have been shown to have a negative effect on the salinity tolerance of fishes (Al Amoudi et al. 1996, Allanson and Bok 1971, Staurnes et al. 2001). Tilapia are considered eurythermal, but their survival has been reduced when they are exposed to water temperatures that are representative of the Salton Sea during the winter (Al Amoudi et al. 1996, Allanson and Bok 1971, Sardella et al. 2004a). Changes in temperature can result in instantaneous and dramatic changes in metabolism, membrane fluidity and integrity, and enzyme function, all of which can negatively affect osmoregulation (Al Amoudi et al. 1996, Robertson and Hazel 1999, Almansa et al. 2003). We hypothesize that temperature-induced tilapia mortality during winter months at the Salton Sea results from osmoregulatory failure.

The purpose of this study was to determine the effect of temperature change on the osmoregulatory ability of 'California' Mozambique tilapia, in order to investigate whether temperature-induced osmoregulatory disturbances may be a contributing factor to winter die-offs. Fish were acclimated to 43 g/l (the current Salton Sea salinity) at 25°C before transfer to 15 or 35 °C for 5 d. Plasma osmolality, [Na^+], and [Cl^-], muscle

water content, and branchial Na^+, K^+-ATPase (NKA) were measured at 0, 24, and 120 h following transfer. A second experiment was carried out in order to demonstrate how temperature can negatively affect what is a mild salinity challenge for this species (Sardella et al. 2004b). In this experiment fish were acclimated to SW (33 g/l) at 25 °C and then transferred to 43 g/l at 15, 25, or 35 °C. Plasma osmolality and branchial NKA activity were measured in these fish at 0, 3, 6, 12, and 24 h following transfer.

MATERIALS AND METHODS

Experimental Animals

California Mozambique tilapia were donated by Pacific Aquafarms in Niland, CA, and transported to San Diego State University in San Diego, CA, where all experiments were conducted. Fish were reared in freshwater and acclimated to 35 g/l (SW) gradually over 3 weeks (as described by Sardella et al. (2004b)). All tanks were filtered by mechanical, chemical, and biological systems, aerated using submersible air stones, and held at a constant temperature of 25 °C. Fish used in both experiments weighed on average 30.18 ± 0.75 g. Salinity was manipulated by adding Instant Ocean synthetic sea salts[1], and measured by light refractometery.

Experiment 1: Temperature Transfer

Thirty-five SW-acclimated tilapia were acclimated to a salinity of 43 g/l at 25 °C and for 2 weeks. Following this acclimation temperature was altered from 25 °C to 15 or 35 °C over one approximately hour. Seven fish were sampled prior to transfer, and seven fish were sampled (see below) at each temperature 24 and 120 h following temperature change.

Experiment 2: Temperature Transfer during Simultaneous Salinity Transfer

After 3 weeks of acclimation to SW at 25°C, seven CA Mozambique tilapia were sampled. Twenty-eight SW-acclimated tilapia were directly transferred from SW at 25°C to 43 g/l at 25 °C, with seven fish being removed and sampled (see below) at 3, 6, 12, and 24 h following transfer. This transfer and sampling protocol was then repeated with transfers from SW at 25 °C to 43 g/l at either 15 or 35 °C.

[1]We used Instant Ocean Synthetic Sea Salt to manipulate salinities in this experiment. It should be noted that the ionic composition of the Salton Sea differs from Instant Ocean at common salinity; Salton Sea water contains approximately two-fold calcium ion and three-fold sulphate ion. In previous experiments (Sardella 2006), it was shown the effects of these differences were negligible with respect to the osmoregulatory ability of tilapia.

Sampling

Fish were sacrificed by a lethal dose of benzocaine anesthetic, previously dissolved in 70% ethanol, and then diluted to a final concentration of 0.7 g/l. The caudal peduncle was severed, and blood was collected from the caudal vein into heparinized microhematocrit centrifuge tubes, which were subsequently centrifuged in a Damon IEC MB microhematocrit centrifuge, and plasma was expelled into Microcentrifuge tubes. In experiment 1 plasma osmolality was measured using a Wescor 5500 vapor pressure osmometer (Wescor Inc. Logan, Utah, USA), plasma [Cl^-] was measured using the colorimetric mercuric thiocyanate method (Zall et al. 1956), and plasma [Na^+] was measured with an atomic absorption spectrophotometer (Perkin Elmer model 3100 A). Muscle water content was determined by removal of approximately 1 g of posterior epaxial muscle, which was rinsed and patted dry, then placed into a pre-weighed scintillation vial and dried in a 70 °C oven. The difference in weight was expressed as a percentage of original wet weight. Lastly, the second and third left gill arches were collected, frozen quickly on dry ice, and stored at -80 °C for later analysis of NKA activity.

Na^+, K^+-ATPase Activity

Gills were homogenized in ~1 ml of SEID buffer (250 mM sucrose, 10 mM EDTA×Na_2, 50 mM imidazole, pH 7.3, deoxycholic acid (0.05%). Branchial Na^+, K^+-ATPase activity was determined as described by McCormick (1993) and expressed as µMol of ADP per hour per µg total protein. Protein was measured using the Biuret method.

Statistical Analyses

A two-way analysis of variance (ANOVA) test was used to determine the effects of temperature and time of exposure on all parameters. A post hoc Holm-Sidak multiple means comparison test was performed when two-way ANOVA results were significant. Statistical tests were performed using SigmaStat version 3.0, with an $\propto$ value of 0.05 ($n = 7$).

RESULTS

Experiment 1: Temperature Transfer

In 43 g/l salinity-acclimated fish, transfer to 15 or 35 °C did not result in loss of orientation or mortality but several sub-lethal effects were observed. Two-way ANOVA results for plasma osmolality, [Na^+], and [Cl^-] following transfer are presented in Tables 1-3. Both osmolality and [Na^+] were significantly increased at 24 h in 15 °C water, and osmolality was reduced to near pre-transfer values after 120 h (Fig. 1a). In contrast, plasma [Na^+] decreased from the peak value at 24 h to an elevated but stable level (Fig. 1b). Plasma osmolality in 35 °C-exposed fish was significantly increased 120

Table 1. Two-way ANOVA results for plasma osmolality following temperature transfer.

Factor	DF	SS	F	*P*
Time	2	15161.5	18.76	< 0.001
Temperature	1	1620.7	4.01	0.054
Interaction	2	7529.1	9.31	< 0.001
Residual	32	12932.7		
Total	37	36436.7		

Table 2. Two-way ANOVA results for plasma chloride following temperature transfer.

Factor	DF	SS	F	*P*
Time	2	3217.5	59.68	< 0.001
Temperature	1	416.4	15.40	< 0.001
Interaction	2	496.5	9.20	< 0.001
Residual	33	889.5		
Total	38	4863.5		

Table 3. Two-way ANOVA results for plasma chloride following temperature transfer.

Factor	DF	SS	F	*P*
Time	2	699.11	8.28	0.001
Temperature	1	186.72	4.28	0.047
Interaction	2	160.92	1.91	0.165
Residual	33	1349.99		
Total	38	2351.24		

h following transfer, but plasma [Na^+] was significantly increased at both 24 and 120 h. Two-way ANOVA results for muscle water content are presented in Table 4. Muscle water content was reduced 24 h following transfer and recovered by 120 h (Fig. 2), but there was no difference between temperatures. Lastly, there were no significant changes in branchial NKA activity at 24 or 120 h following temperature transfers (Pooled Value = 7.69 ± 0.62 μmol/h/μg protein; data not shown).

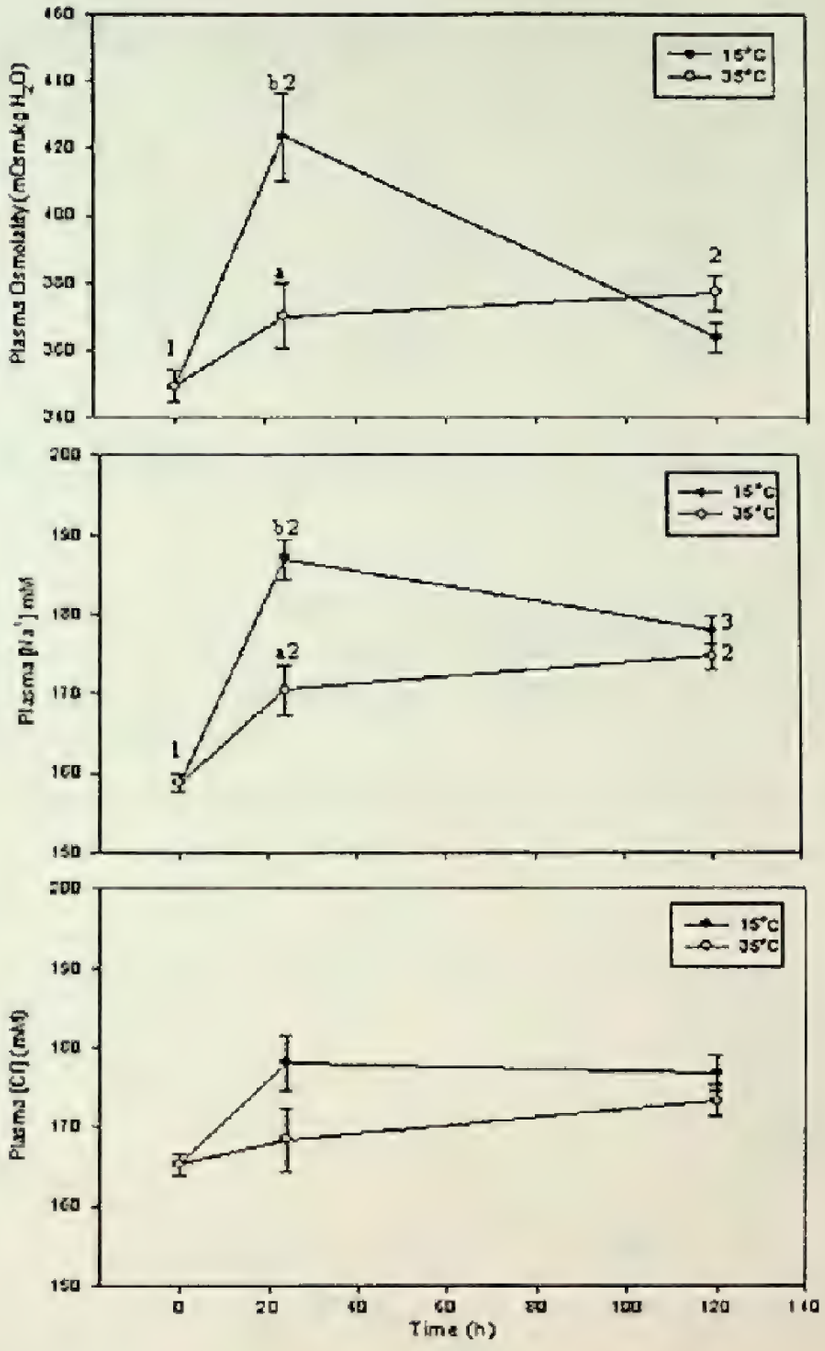


Figure 1. The effect of a ±10 °C temperature change on a) plasma osmolality, b) plasma [Na^+], and c) plasma [Cl^-] in California Mozambique tilapia acclimated to 43 g/l salinity. Letters indicate significant differences between fish at 15 °C compared to 35 °C at a given time, whereas numbers denote significant differences relative to time zero within a given temperature as determined by two-way ANOVA ($p<0.001$).

Experiment 2: Temperature Transfer during Simultaneous Salinity Transfer

There was no mortality or loss of equilibrium in tilapia transferred from SW at 25 °C to 43 g/l salinity at 25 or 35 °C. However, when fish were transferred to 43 g/l at 15 °C, there was 100% mortality after 12 h. Two-way ANOVA results for plasma osmolality following a simultaneous salinity and temperature transfer are presented in Table 5. In

Table 4. Two-way ANOVA results for muscle water content following temperature transfer.

Factor	DF	SS	F	*P*
Time	2	102.38	6.132	0.005
Temperature	1	15.48	1.855	0.182
Interaction	2	8.96	0.537	0.590
Residual	34	283.86		
Total	39	415.21		

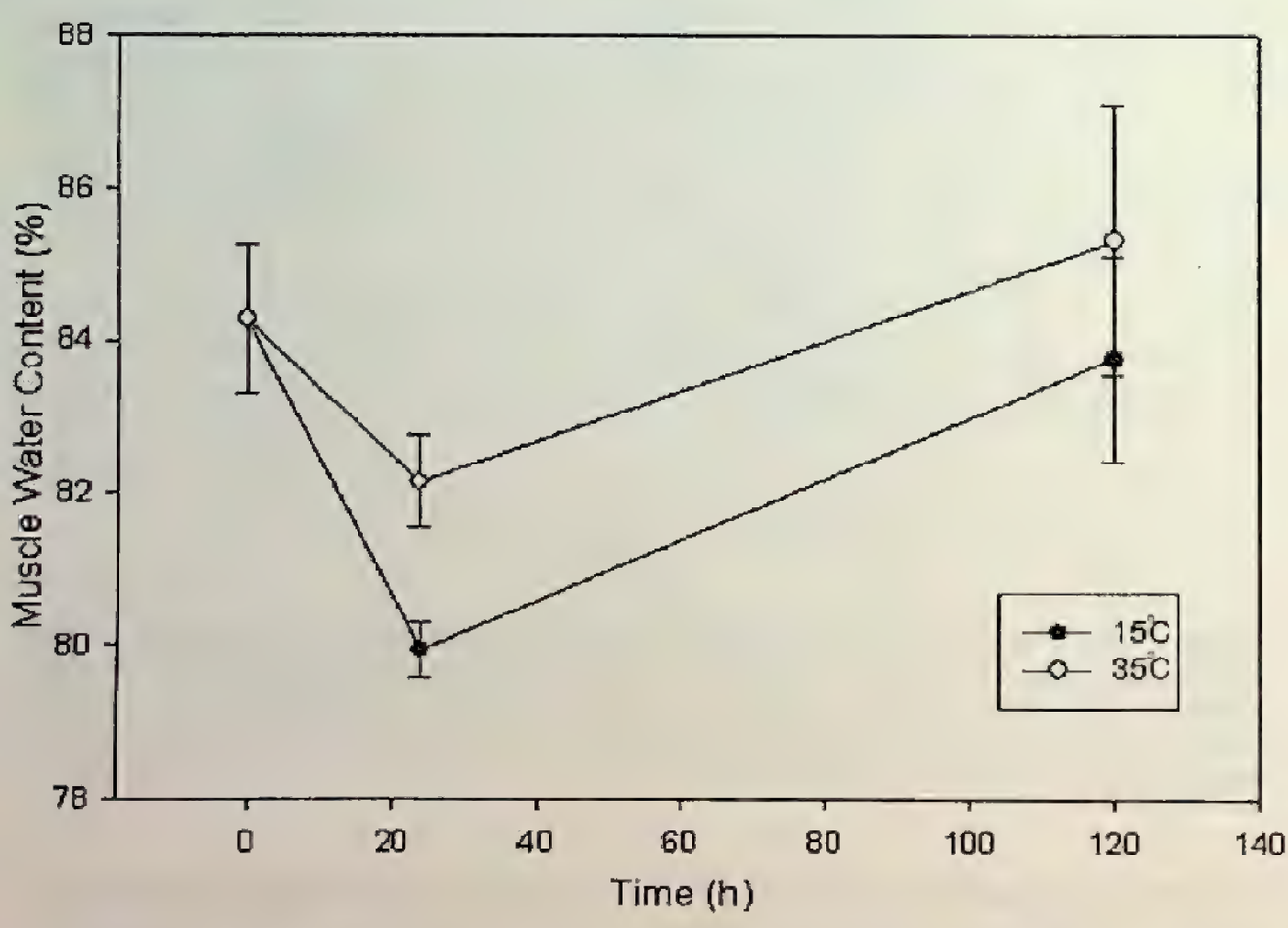


Figure 2. The effect of a ±10 °C temperature change on muscle water content in California Mozambique tilapia acclimated to 43 g/l salinity. There was a significant effect of time as determined by two-way ANOVA ($p<0.01$).

Table 5. Two-way ANOVA results for plasma osmolality following salinity and temperature transfer.

Factor	DF	SS	F	*P*
Time	3	358081	127.9	< 0.001
Temperature	2	47440	25.43	< 0.001
Interaction	6	568235	101.5	< 0.001
Residual	54	59712		
Total	75	1096386		

fish transferred to 15 °C, plasma osmolality increased at each time point up to 12 h, while osmolality in fish at 35 °C was significant increased at 6 h after transfer and remained elevated over 24 h. In contrast, fish transferred to 25 °C maintained plasma osmolality over the entire experiment (Fig. 3).

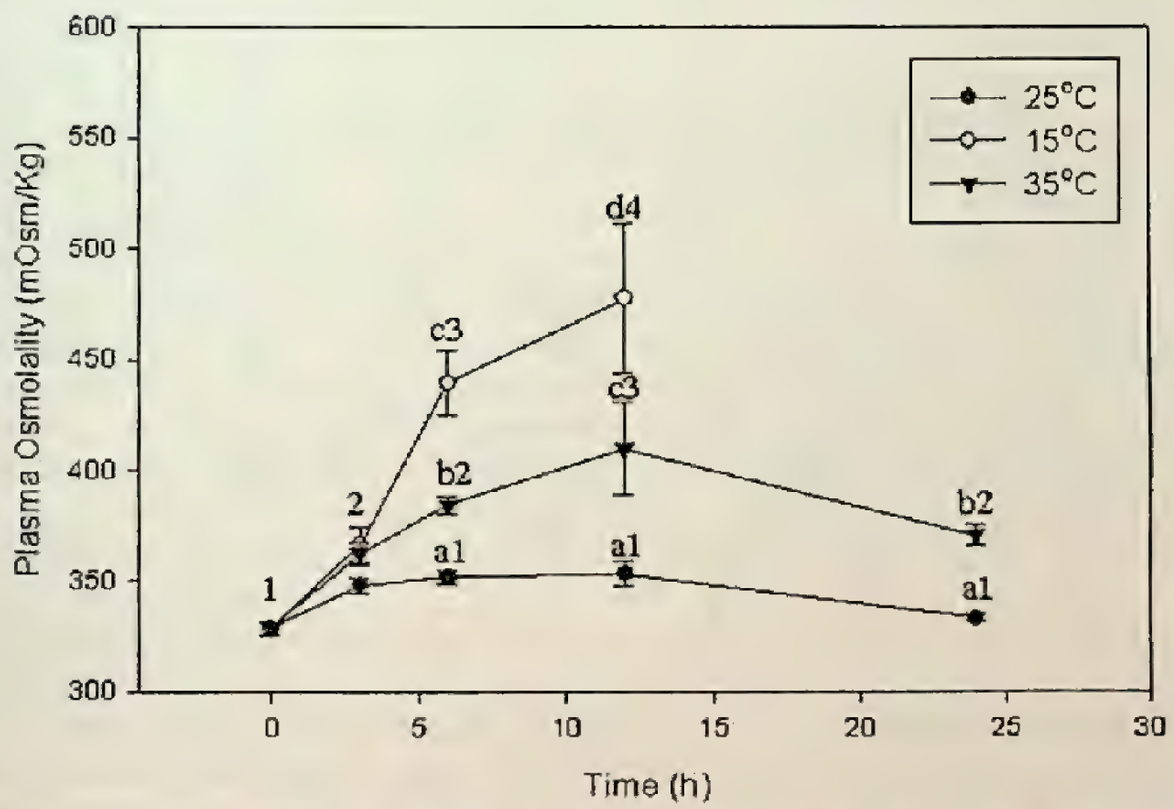


Figure 3. The effect of transfer from 35 to 43 g/l salinity at 15, 25, and 35 °C on plasma osmolality in California Mozambique tilapia. Letters indicate significant differences due to temperature at a given time, whereas numbers denote significant differences relative to time zero within a given temperature as determined by two-way ANOVA ($p<0.001$).

Two-way ANOVA results for branchial NKA activity following simultaneous salinity and temperature transfer are presented in Table 6. NKA activity from tilapia transferred at 25 °C was elevated at 3 h and remained high at 12 h before beginning to decline by 24 h (Fig. 4). The fish transferred at 15 and 35 °C also showed an increase in NKA activity at 3 h but in these groups, it decreased to pre-transfer levels by 6 h. While NKA from 15 °C-acclimated tilapia remained low up until the death of the fish, NKA activity in the 35 °C-acclimated fish showed a second increase that was significantly higher than pre-transfer values by 24 h.

Table 6. Two-way ANOVA results for branchial NKA activity following salinity and temperature transfer.

Factor	DF	SS	F	*P*
Time	4	8.6	5.96	< 0.001
Temperature	2	12.66	17.54	< 0.001
Interaction	8	22.25	7.71	< 0.001
Residual	86	31.03		
Total	100	77.80		

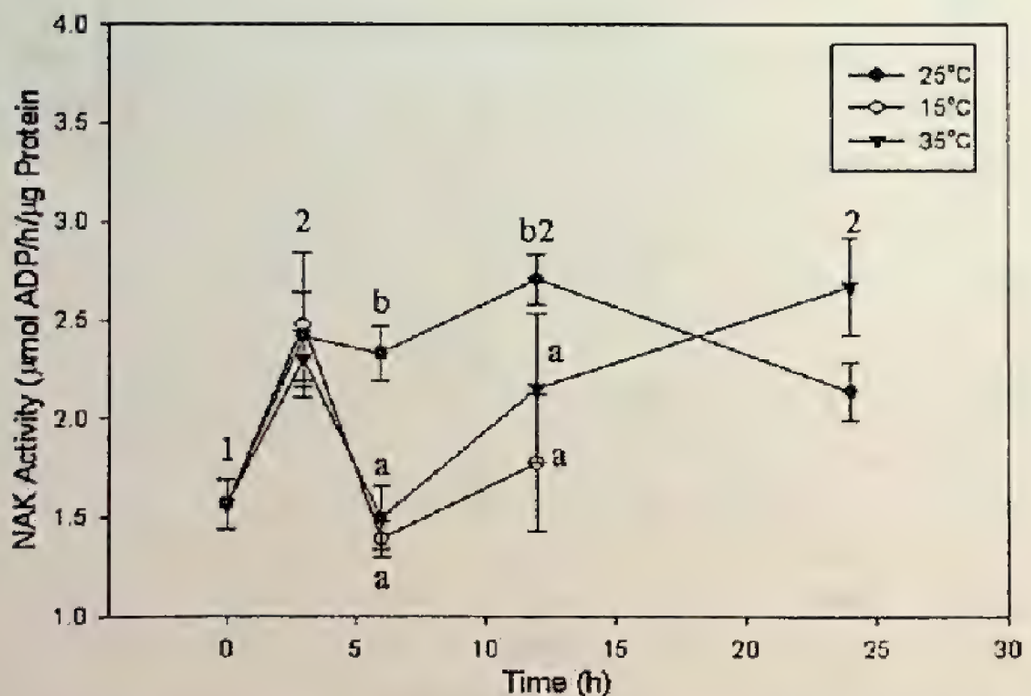


Figure 4. The effect of transfer from 35 to 43 g/l salinity at 15, 25, and 35 °C on branchial NKA activity in California Mozambique tilapia; all assays were carried out at 25 °C. Letters indicate significant differences due to temperature at a given time, whereas numbers denote significant differences relative to time zero within a given temperature as determined by two-way ANOVA ($p<0.01$).

DISCUSSION

All Salton Sea fish are subject to mortality events but during the winter months, the bulk of the fish kills has been tilapia (Hurlbert et al. 2007). Based on the results of this study, the effects of reduced temperature on osmoregulatory ability is potentially a large contributor to the high winter mortality. Our results agree with previously published work showing that the effects of the interaction between temperature and salinity pose a greater challenge than the application of either stress alone (Al Amoudi et al. 1996, Allanson and Bok 1971, Sardella et al. 2004a).

The goal of experiment 1 was to demonstrate the effect of a temperature transfer carried out at a constant salinity. The response of tilapia to the temperature change was similar to what has been observed following a salinity increase from 35 to 60 g/l at 25 °C, with large increases in both plasma osmolality and [Na^+] (Sardella et al. 2004b; Fig. 1). These changes indicate an immediate loss of osmotic balance following the transfer. The increased osmolality was correlated with decreased muscle water content at 24 h (Fig. 2), as the high osmotic pressure draws water out of the intracellular compartment. Muscle water loss in this species does not occur following salinity increases at 25 °C, even when salinities are as high as 95 g/l and plasma osmolality reaches levels greater than 400 mOsm/kg H_2O (Sardella et al. 2004b). Loss of muscle water in this study indicates a potential role for temperature in the loss of cellular water, as the levels of osmolality in this study, while high, have not been shown to decrease muscle water previously. Temperature has been shown to have detrimental effects on salt and water balance in that it can alter enzyme kinetics, disrupt membrane dynamics, and alter the diffusion gradient across the gill due to compromises between osmoregulation and respiration confounded by changes in metabolic rate (Randall et al. 1972, Gonzalez and McDonald 1992, Hochachka and Somero 2002). One hundred and twenty hours following temperature transfer there was no difference in plasma osmolality between fish transferred to 15 versus 35 °C, but the temporary disturbance at 24 h was greater in 15 °C exposed fish, indicating that decreases in temperature are more detrimental than increases over this range. These data were consistent with previous studies with this species (Allanson and Bok 1971, Al Amoudi et al. 1996, Sardella et al. 2004a). While it appeared that tilapia were able to acclimate to the temperature changes by 120 h in this laboratory experiment, within the Salton Sea where they must actively forage and avoid predation, as well as deal with several other environmental stressors, their threshold for salinity tolerance at 15 °C could be dramatically reduced.

Although an abrupt temperature change represents a worse-case scenario with respect to its effects on osmoregulation, we exposed tilapia to a combined temperature and salinity challenge for 24 h based on previous findings that the first 12 h are the most crucial for tilapia acclimation (Hwang 1987, Hwang et al. 1989). Acute 24 h challenges are commonly used to assess overall fitness and have been shown in other species to be well representative of osmoregulatory ability (Brauner et al. 1992, Clarke and Shelbourn 1982, Clarke and Blackburn 1977). Tilapia were able to maintain osmotic balance following a 10 g/l increase in salinity at constant temperature for up to 24 h (Fig.

3), with minimal yet significant increases in branchial NKA activity at 12 h (Fig 4). Twelve hours has been previously described as the end of the 'crisis period' for this species, which represents the timing of the peak dehydration that must be compensated for by the fish while longer-term osmoregulatory capacity is upregulated (Hwang et al. 1989). Tilapia did not maintain plasma osmolality at the same level when temperature was increased to 35 °C; plasma osmolality increased at 3 h following transfer, peaked at 12 h, and was still significantly elevated at 24 h (Fig. 3). Again, the peak in osmolality coincided with what Hwang et al. (1989) described as the crisis period during salinity acclimation. The increased osmolality in fish held at 35 °C relative to 25 °C values is likely associated with the temperature-induced increase in metabolic rate (Q_{10} effects). Under these conditions, gill water and blood flow become elevated to meet the demands of metabolism, which subsequently increases the potential for osmotic disequilibration across the gill epithelium (Randall et al. 1972, Gonzalez and McDonald 1992). The enhanced osmotic gradient resulting from hypermetabolism offsets any advantage of increased gill NKA activity (Hochachka and Somero 2002, Sardella et al. 2004a).

Fish transferred from 35 g/l salinity at 25 °C to 43 g/l salinity at 15°C did not survive past 12 h (the crisis period). Prior to death, fish from this treatment group had steadily increasing plasma osmolality (Fig. 3). The likely cause of mortality was loss of osmoregulatory control (Allanson and Bok 1971, Al Amoudi et al. 1996), as NKA activity has been shown to be dramatically reduced when assayed at 15 °C (Sardella et al. 2004a). These data provide a clear demonstration of how an additionally-imposed stress can complicate what has been previously observed to be a simple salinity acclimation.

Tilapia in all temperatures showed an initial increase in NKA at 3 h, but the fish with the additional imposed temperature stress were unable to maintain the elevated activity (Fig. 4), which may have ultimately led to elevated plasma osmolality. A rapid activation of gill NKA has been observed in this Mozambique tilapia (Hwang et al. 1989, Sunny and Ommen 2001) as well as the common killifish (*Fundulus heteroclitus*) (Towle et al. 1977, Mancera and McCormick 2000), which is also a well known model of euryhalinity.. The cause behind the inability to sustain NKA activity in 15 and 35 °C water is unclear and provides an interesting course for further study.

The results clearly show that a temperature change can have a profound effect on the osmoregulatory ability of California Mozambique tilapia, and that a combined temperature and salinity challenge was more stressful than either stress applied alone. Tilapia exposed to 15 °C in this and previous studies have been shown to experience osmoregulatory difficulty, and temperature within the Salton Sea during the winter months has been observed to decrease in some years below 15 °C (Watts et al., 2001). While temperature changes in the Salton Sea are more gradual relative to laboratory experiments, these data provide a good example off how temperature shifts can negatively affect osmoregulation. We have only investigated two of the multiple stressors that exist in the Salton Sea. Additional simultaneous stressors may have further additive effects, for example in a previous study, tilapia were acclimated to water collected from the Salton Sea proper at 25 °C, and when the temperature was reduced to 15 °C, 100% mortality resulted in as little as 3 h (Sardella 2006).

The documented use of river mouth areas within the Salton Sea by tilapia during periods of low temperature (Hurlbert et al. 2007) could possibly represent an attempt to alleviate temperature-induced osmoregulatory disturbances, but direct evidence to confirm this phenomenon has yet to be collected. Further investigation into the possible selection of lower salinities when faced with a temperature stress would serve as a test of this hypothesis. Based on experimental findings from this and other studies involving abrupt temperature challenges, it is reasonable to conclude that osmoregulatory failure during the winter cold is a major contributor to tilapia mortality in the Salton Sea.

ACKNOWLEDGMENTS

We would like to say thanks to many people who made this study possible. First and foremost, to B. Engler and C. Bornia of Pacific AquaFarms in Niland, CA for their generous donation of fish for this and other studies. We also like to thank R. Gonzalez and J. Cooper of the University of San Diego, as well as E. Dale and P. Davis of San Diego State University. For additional help and support, we thank J. Crayon of the CA Department of Fish and Game, as well as R. Stendell and D. Barnum of the Salton Sea Science Committee. This work was financially supported by the Salton Sea Authority, as well as an NSERC Discovery Grant to CJB.

LITERATURE CITED

Al Amoudi, M., A. El-Sayed, and A. Ghobashy. 1996. Effects of Thermal and Thero-haline shocks on survival and osmotic concentration of the tilapias *Oreochromis mossambicus* and *Oreochromis aureus x Oreochromis niloticus* Hybrids. Journal of the World Aquaculture Society 27:456-461.

Allanson, B.R., and A. Bok. 1971. The influence of exposure to low temperature on *Tilapia mossambica* Peters (Cichlidae). Journal of Fish Biology 3:181-185.

Almansa, E., J.J. Sanchez, S. Cozzi, C. Rodriguez, and M. Diaz. 2003. Temperature-activity relationship for the intestinal Na^+-K^+-ATPase of *Sparus aurata*. A role for the phospholipid environment? Journal of Comparative Physiology B 173:231-237.

Brauner, C. J., J. M. Shrimpton, and D. J. Randall. 1992. Effect of short-duration seawater exposure on plasma ion concentrations and swimming performance in Coho salmon (*Oncorhychus kisutch*) parr. Canadian Journal of Fisheries and Aquatic Sciences 49: 2399-2405

Clarke, W. C. and J. Blackburn. 1977. A seawater challenge test to measure smolting of juvenile salmon. Canadian Fisheries and Marine Service Technical Report, 11pp.

Clarke, W. C. and J. E. Shelbourn. 1982. Growth and smolting of under-yearling Coho salmon in relation to photoperiod and temperature. Proceedings of the Northern Pacific Aquacultural Symposium. August 1980, Anchorage, AK. Alaska Sea Grant Grant Rep. 82(2): 200-216.

Costa-Pierce, B.A., and R.W. Doyle. 1997. Genetic identification and status of tilapia regional strains in southern California., p. 1-17, *In* B. A. Costa-Pierce and J. E. Rakocy, eds. World Aquaculture, Vol. 1. Worlds Aquaculture Society, Baton Rouge, LA.

Gonzalez, M.R., C.M. Hart, J.R. Verfaillie, and S.H. Hurlbert. 1998. Salinity and fish effects on Salton Sea microecosystems: Water chemistry and nutrient cycling. Hydrobiologia 381:105-128.

Gonzalez, R.J., and D.G. McDonald. 1992. The relationship between oxygen consumption and ion loss in a freshwater fish. Journal of Experimental Biology 163:317-332.

Hanson, J.A. 1970. Salinity tolerances for Salton Sea fishes. Department of Fish and Game 70:2-7.

Hochachka, P.W., and G.N. Somero. 2002. Biochemical Adaptation: Mechanism and Process in Physiological Evolution Oxford University Press, New York, NY.

Hurlbert, A. H., T. Anderson, K. K. Sturm, and S. H. Hurlbert. 2007. Fish and fish-eating birds at the Salton Sea: a century of boom and bust. Hydrobiologia (in press).

Hwang, P., C.M. Sun, and S.M. Wu. 1989. Changes of plasma osmolarity, chloride concentration and gill Na^+-K^+-ATPase activity in tilapia (*Oreochromis mossambicus*) during seawater acclimation. Marine Biology 100:295-299.

Hwang, P.P. 1987. Tolerance and ultrastructural responses of branchial chloride cells to salinity changes in the euryhaline teleost *Oreochromis mossambicus*. Marine Biology 94:643-649.

Mancera, J.M., and S.D. McCormick. 2000. Rapid activation of gill Na^+/K^+-ATPase in the euryhaline teleost *Fundulus heteroclitus*. Journal of Experimental Zoology 287:263-274.

McCormick, S.D. 1993. Methods for non-lethal gill biopsy and measurement of Na^+, K^+-ATPase acticity. Journal of Fisheries and Aquatic Sciences. 50: 656-658

Randall, D.J., D. Baumgarten, and M. Malyusz. 1972. The relationship between gas and ion transfer across the gills of fishes. Comparative Biochemistry and Physiology 41a:629-637.

Riedel, R., L. Caskey, and B.A. Costa-Pierce. 2002. Fish biology and fisheries ecology of the Salton Sea. Hydrobiologia 473:229-244.

Robertson, J.C., and J.R. Hazel. 1999. Influence of temperature and membrane lipid composition on the osmotic water permeability of teleost gills. Physiological and Biochemical Zoology 75:623-632.

Sardella, B. 2006. The limits of osmoregulation: strategies for tolerance and acclimation of California Mozambique tilapia to conditions of the Salton Sea. Ph.D. Dissertation. University of British Columbia, Vancouver, BC Canada.

Sardella, B., J. Cooper, R. Gonzalez, and C.J. Brauner. 2004a. The effect of temperature on juvenile Mozambique tilapia hybrids (*Oreochromis mossambicus* x *O. urolepis hornorum*) exposed to full-strength and hypersaline seawater. Comparative Biochemistry and Physiology A 137:621-629.

Sardella, B., V. Matey, J. Cooper, R.J. Gonzalez, and C.J. Brauner. 2004b. Physiological, biochemical, and morphological indicators of osmoregulatory stress in California Mozambique tilapia (*Oreochromis mossambicus* x *O. urolepis hornorum*) exposed to hypersaline water. Journal of Experimental Biology 207:1399-1413.

Sardella, B. A., V. Matey and C. J. Brauner. 2007. Coping with multiple stressors: physiological strategies and mechanisms in Salton Sea fishes. Hydrobiologia, in press.

Staurnes, M., T. Sigholt, T. Asgard, and G. Baeverfjord. 2001. Effects of a temperature shift on seawater challenge test performance in Atlantic salmon (*Salmo salar*) smolt. Aquaculture 201:153-159.

Stickney, R.R. 1986. Tilapia tolerance of saline waters: a review. The Progressive Fish Culturalist 48:161-167.

Sunny, F., and O.V. Oommen. 2001. Rapid action of glucocorticoids on branchial ATPase activity in *Oreochromis mossambicus*: an in vivo and in vitro study. Comparative Biochemistry and Physiology B 130:323-330.

Towle, D.W., M.E. Gilman, and J.D. Hempel. 1977. Rapid modulation of gill sodium and potassium dependent ATPase activity during acclination of the killifish (*Fundulus heteroclitus*) to salinity change. Journal of Experimental Zoology 202:179-186.

Walker, B.W., R. R. Whitney, and G. W. Barlow. 1961. The fishes of the Salton Sea. In B. W.

Walker (ed.), The ecology of the Salton Sea, California in relation to the sportfishery. California Department of Fish and Game, Fish Bulletin 113: 77-91

Watts, J.B., B. Swan, M.A. Tiffany, and S. Hurlbert. 2001. Thermal mixing, and oxygen regimes of the Salton Sea, California, 1997-1999. Hydrobiologia 466:159-176.

Zall, D.M., D. Fisher, and M.D. Garner, 1956. Photometric determination of chlorides in Water. Analytical Chemistry 28: 1665-1678.

Received: 27 September 2006
Accepted: 7 March 2007

California Fish and Game 93(1):214-223 2007

RELATION BETWEEN MORTALITY OF PRICKLY SCULPIN AND DIURNAL EXTREMES IN WATER QUALITY AT RODEO LAGOON, MARIN COUNTY, CALIFORNIA

BARBARA A. MARTIN and MICHAEL K. SAIKI
U.S. Geological Survey
Biological Resources Division, Western Fisheries Research Center, Dixon Duty Station
6924 Tremont Road
Dixon, California 95620
barbara_ann_martin@usgs.gov

DARREN FONG
National Park Service
Golden Gate National Recreation Area
Building 1061, Fort Cronkhite
Sausalito, California 94965

INTRODUCTION

Occasional fish die-offs occur during fall months (September-November) in Rodeo Lagoon and the adjoining Rodeo Lake in Marin County, California. Prickly sculpin (*Cottus asper*) usually constitute the largest numbers of dead individuals (Fong 1997[1]). The occurrence of dead fish often coincides with the senescence phase of algal blooms or the seasonal dieback of submerged macrophytes, events that can create potentially stressful water quality conditions for fish. However, lack of information on the water quality tolerances of prickly sculpin and other affected fishes have stymied efforts to conclusively identify the causative factors responsible for fish die-offs.

The purpose of this study was to develop a better understanding of the cause of acute mortality in prickly sculpin inhabiting Rodeo Lagoon and Rodeo Lake. Specific objectives were to determine if mortality varied among sites and seasons, and if mortality was associated with high water temperature, low dissolved oxygen concentration, high pH, high salinity concentration, high turbidity, high un-ionized ammonia concentration, or a combination of these variables.

DESCRIPTION OF THE STUDY AREA

Rodeo Lagoon and Rodeo Lake are located in the Golden Gate National Recreation Area north of San Francisco (Fig. 1). Rodeo Lagoon is a small (15.2 ha), shallow (1-2 m deep in late fall), brackish water body separated by a concrete weir from the smaller (1.0 ha) and fresher Rodeo Lake. Although the lagoon receives inflow from the lake

[1]Fong, D. 1997. 1996 fall fish kill evaluation for Rodeo Lagoon, Golden Gate National Recreation Area, Marin Co. Prepared for the Division of Resource Management and Planning, Golden Gate National Recreational Area. 9 p.

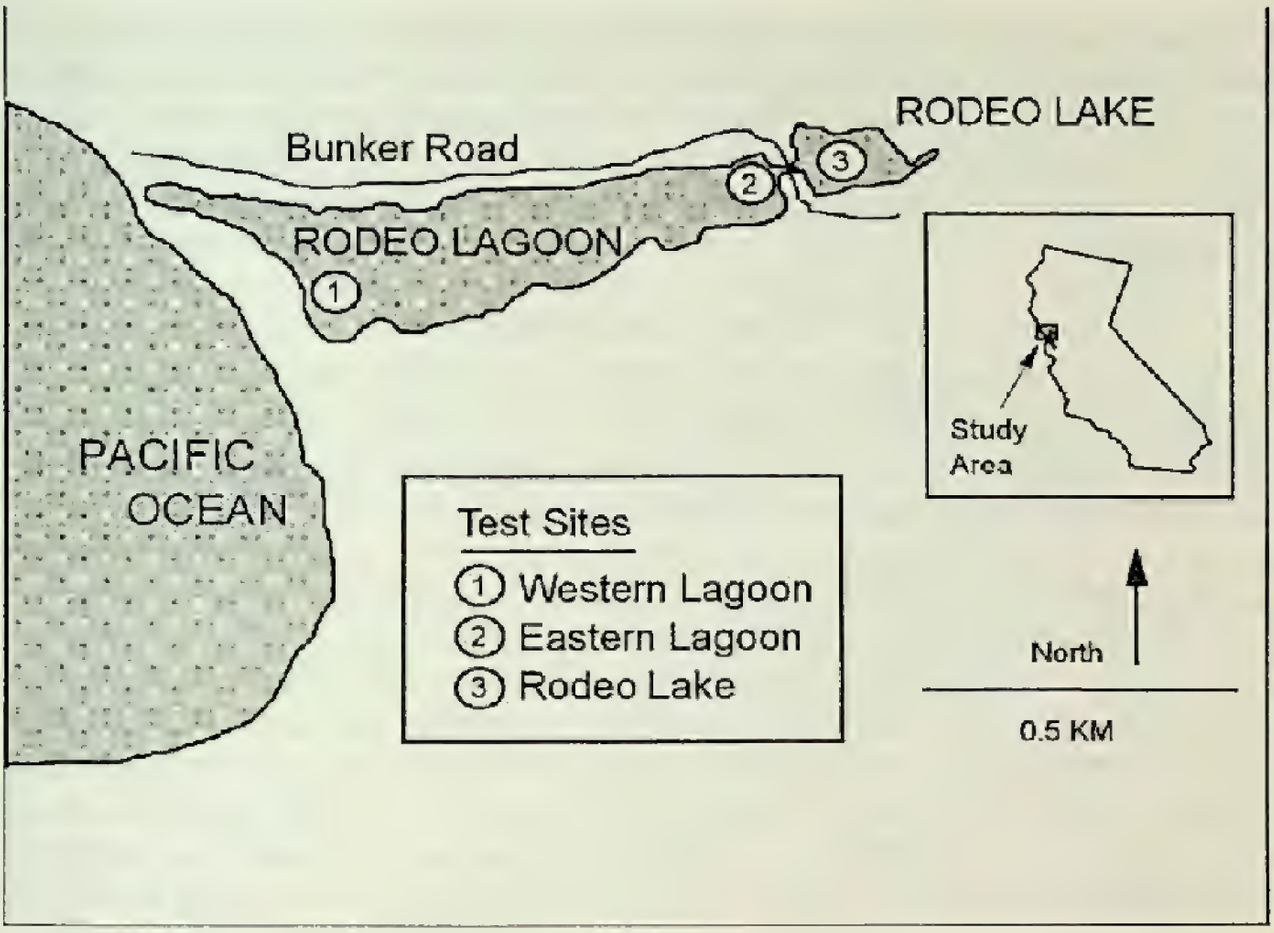


Fig. 1. Map of the study area showing the locations of three test sites.

(which, in turn, is fed by surface runoff and springs in the watershed), it is a closed system for most of the year (Wang 1984[2]). A sand bar at the seaward end of the lagoon breaches irregularly and for only brief periods during winter months in response to erosion from high water levels in the lagoon (caused by heavy rainfall) and strong wave action from the Pacific Ocean. Available studies suggest that water quality between Rodeo Lagoon and Rodeo Lake differs mostly in terms of salinity (Wang 1984[2], Podlech et al. 1993[3], Codemo et al. 1996[4], Beutel 1998[5]).

---

[2]Wang, J.C.S. 1984. On the ecological status of the tidewater goby, *Eucyclogobius newberryi* (Girard), in a lagoon and lake of the Golden Gate National Recreation Area, CA. Technical Report No. 15, Cooperative National Park Resources Study Unit, University of California, Davis. 25 p.

[3]Podlech, M., C.M. Codemo, R.J. Brown, & W.P. Jordan. 1993. Some physical, chemical, and biological characteristics of Rodeo Lagoon, Rodeo Lake, and Rodeo Creek - initial study. Prepared for the National Park Service, Golden Gate National Recreational Area, 29 p. + appendices.

[4]Codemo, C.M., M. Podlech, R.J. Brown, & W.P. Jordan. 1996. Characterization of phytoplankton in Rodeo Lagoon and Rodeo Lake, GGNRA, during August 1996. Prepared for the National Park Service, Golden Gate National Recreation Area, 14 p. + appendices.

[5]Beutel, M. 1998. Golden Gate National Recreation Area storm water monitoring program 1997/1998. Prepared for the National Park Service, Golden Gate National Recreational Area Contract Number 1443PX8140-97-251, 41 p. + appendices.

During late summer and early fall, Rodeo Lagoon and Rodeo Lake experience heavy infestations of algae and submerged rooted macrophytes. Blue-green algae such as *Nodularia*, *Microcystis*, and *Anabaena*, often dominate in the lagoon whereas the green alga, *Oedogonium*, dominates in the lake (Podlech et al. 1993[3]; Codemo et al. 1997[4]). According to Swenson (1994[6]), *Potamogeton* is the predominant rooted macrophyte.

MATERIALS AND METHODS

We conducted 24-hour-long field tests on nine occasions in fall (15 September - 1 October 1998) and three occasions in winter (12-15 January 1999) to assess acute mortality in prickly sculpin and its relation to selected water quality variables. Using historical water quality data from Rodeo Lagoon and Rodeo Lake (e.g., Wang 1984[2], Podlech et al. 1993[3], Codemo et al. 1996[4], Beutel 1998[5]), we chose water quality parameters for measurement that exceeded criteria for protecting freshwater or estuarine fish. The tests occurred at two sites in Rodeo Lagoon and one site in Rodeo Lake (Fig. 1). Geospatial coordinates for the sites were as follows: 1) west-end of Rodeo Lagoon, 37°49'47.8"N, 122°32'4.3"W; 2) east-end of Rodeo Lagoon, 37°49'54.6"N, 122°31'36.3"W; and 3) west-end of Rodeo Lake, 37°49'55.5"N, 122°31'29.2"W. These sites were chosen to encompass a range of water quality values, with higher dissolved oxygen concentrations typically occurring in the windward side (west end) of Rodeo Lagoon than in the leeward side (east end) of the lagoon and lake. Similarly, winter and fall seasons were selected because of expected differences in water quality conditions.

Two live cages containing prickly sculpin and a Hydrolab DataSonde®3 multiprobe logger (Hydrolab Corporation, Austin, Texas) were installed at each of the three test sites. The live cages were constructed from 18.9-L plastic buckets modified to allow water circulation by cutting two 34-cm x 23-cm openings in the sides, then covering the openings with 0.043-cm mesh stainless steel screen (Martin and Saiki 1999). At each lagoon site, three 2-m steel fence posts were placed in a triangular pattern (each post about 1 m away from the other two posts). The cages were suspended from two posts so that each cage rested near the bottom. The Hydrolab DataSonde®3 multiprobe logger was secured to the 3rd post with probes about 0.1 m from the bottom. At the lake site, live cages tethered with 1-m lengths of galvanized cable to styrofoam buoys were positioned so that the cages rested near the bottom. The styrofoam buoys were attached to a large air-filled main buoy which, in turn, was attached by a cable to a 4.5-kg anchor. A multiprobe logger was attached to the cable of the main buoy so that the probes were suspended about 0.1 m from the bottom.

Adult prickly sculpin averaging 70 mm in standard length (SL; 95 % confidence interval, 68-72 mm) and 8.3 g in weight (95 % confidence interval, 7.5-9.1 g) were collected daily (7 am to 10 am) from the eastern portion of Rodeo Lagoon using a 3.2 mm-mesh bag seine or several 3.2 mm-mesh unbaited minnow traps. Immediately following capture, sculpins were inspected and roughly sorted for size (only healthy fish larger

[6]Swenson, R.O. 1994. A survey of the tidewater goby (*Eucyclogobius newberryi*) at Rodeo Lagoon, Marin County, California. Department of Integrative Biology, University of California, Berkeley, unpublished final report, 14 p.

than about 50 mm SL were retained), then placed into one of six aerated 18.9-L plastic buckets (10-12 fish/bucket) containing site water and some aquatic vegetation (for cover). To help maintain relatively constant water temperatures during fish collections and while awaiting transport to a test site, the buckets were kept partially submerged in shallow water, with at least half of the volume renewed at roughly 30-minute intervals. Sculpins were not collected from Rodeo Lake because they were too rare at this locality to yield the large numbers needed for our tests. After completing the fish collections, test sites were visited in random order with two buckets of fish. Upon arrival at a test site, 10 fish selected at random from the buckets were placed into each live cage. Any remaining fish were then returned to the eastern portion of the lagoon and released alive.

Tests were terminated after prickly sculpin had been exposed to ambient water conditions for about 24 hours. All live and dead fish were counted, then measured for SL and weighed.

Each Hydrolab DataSonde®3 multiprobe logger was calibrated and programmed to take hourly measurements of pH, dissolved oxygen, water temperature, salinity, and turbidity (if a turbidity probe was available). Field accuracy of the Hydrolab measurements was verified by comparing with measurements of daily grab-samples from a YSI model 57 temperature-dissolved oxygen meter (Yellow Springs Instrument Company, Yellow Springs, OH) and a Cole-Parmer model 5994 Digisense pH meter (Cole-Parmer Instrument Company, Chicago, IL). A 0.5-L sample of water was taken from each site to verify turbidity readings using a LaMotte model 2008 Turbidimeter (LaMotte Instrument Company, Chestertown, MD).

Total ammonia was monitored by collecting 0.5-L grab samples of water from each site, acidifying each sample with 6N sulfuric acid, then storing the samples in a freezer for later analysis. Total ammonia was determined with the salicylate method (Walters 1989) using a Hach 2100 spectrophotometer (Hach Company, Loveland, CO). Standard quality control and assurance measures included field and laboratory duplicates, blanks, and known standards. Total ammonia concentrations were converted to un-ionized ammonia concentrations by using the formula in Emerson et al. (1975).

Results were summarized with SAS® statistical software (SAS Institute Inc., Cary, NC). Raw data were either logarithmically transformed or, if expressed in percentages, angular-transformed before undergoing statistical comparisons. Null hypotheses of statistical comparisons were rejected if $P \leq 0.05$.

Stepwise logistic regression was used to assess fish mortality (dependent variable) in relation to the following water quality (independent) variables: maximum temperature, maximum pH, minimum dissolved oxygen concentration, maximum salinity concentration, maximum turbidity, and maximum un-ionized ammonia concentration. The independent variables (maxima or minima) were selected by comparing historical water quality data from Rodeo Lagoon and Rodeo Lake (e.g., Wang 1984[2], Podlech et al. 1993[3], Codemo et al. 1996[4], Beutel 1998[5]) with criteria or other recommendations offered by McKee and Wolf (1963), USEPA (1986), and others for protecting freshwater or estuarine fish, then deciding which variables were most likely to contribute to acute fish mortality. In stepwise logistic regression, the Wald chi-square statistic from the last step of the stepwise algorithm is proportional to the amount of explanatory power

that would be lost by removing a given variable from the model (N.H. Willits, Division of Statistics, University of California, Davis, CA, personal communication). Thus, a water quality variable represented by a large Wald chi-square statistic is more important than other water quality variables represented by smaller Wald chi-square statistics in explaining variations in fish mortality.

RESULTS

All test fish survived 24-hour exposures at the two sampling sites in Rodeo Lagoon during fall and winter. By comparison, high mortality occurred in Rodeo Lake during the fall (complete mortality occurred during six of nine tests whereas partial mortality [55-70 %] occurred during the remaining three tests), but all fish survived during the winter.

Water quality variables exhibited temporal (seasonal) and spatial (site-related)

Table 1. Summary of selected water quality variables in Rodeo Lagoon (western and eastern lagoon sites) and Rodeo Lake during fall 1998 and winter 1999. N, sample size; $\bar{x}$, geometric mean; R, range.

Variable or site	Fall 1998			Winter 1999		
	N	$\bar{x}$	R	N	$\bar{x}$	R
Temperature (°C)						
Western lagoon	216	17.25	15.45-18.70	71	7.49	6.36-8.59
Eastern lagoon	213	17.88	15.48-21.11	71	7.71	6.76-8.28
Rodeo Lake	209	14.36	14.17-14.52	71	7.45	5.51-8.47
Dissolved oxygen (mg/L)						
Western lagoon	216	7.52	5.39-10.39	71	10.43	6.93-11.69
Eastern lagoon	213	9.57	4.56-20.00	71	9.32	6.07-13.5
Rodeo Lake	209	2.46	1.48-7.18	71	10.07	7.63-12.95
pH						
Western lagoon	216	9.36	9.15-9.48	71	8.35	8.22-8.47
Eastern lagoon	213	9.39	9.01-9.69	71	8.13	7.93-8.29
Rodeo Lake	209	7.16	6.98-7.31	71	7.19	7.08-7.35
Salinity (‰)						
Western lagoon	216	2.9	2.6-2.9	71	2.8	2.7-2.8
Eastern lagoon	213	2.8	2.7-2.8	71	2.8	2.7-2.8
Rodeo Lake	209	0.4	0.3-0.4	71	0.1	0.1-0.1
Turbidity (NTU)						
Western lagoon	18	74.3	63.4-87.2	6	15.2	12.0-16.7
Eastern lagoon	18	60.3	35.0-78.4	6	23.4	21.4-25.4
Rodeo Lake	18	15.0	9.2-24.5	6	10.0	9.3-11.0
Un-ionized ammonia (mg/L)						
Western lagoon	8	0.0298	0.0078-0.0631	6	0.0140	0.0125-0.0160
Eastern lagoon	8	0.0214	0.0063-0.0333	6	0.0091	0.0057-0.0132
Rodeo Lake	8	0.0002	0.0001-0.0004	6	0.0009	0.0007-0.0013

patterns (Table 1). Temperature and dissolved oxygen varied greatly between seasons, with higher temperatures and lower dissolved oxygen concentrations occurring in fall than in winter at all sites. Rodeo Lake exhibited exceptionally low dissolved oxygen values in the fall, with concentrations averaging 2.46 mg/L (about 25 % saturation). On average, higher pH levels, salinity concentrations, turbidity levels, and un-ionized ammonia concentrations occurred in Rodeo Lagoon than in Rodeo Lake during both fall and winter.

According to an adjusted Bonferroni P-value of 0.008 (for six simultaneous comparisons), all water quality variables except maximum temperature were significantly correlated with fish mortality (Table 2). However, coefficient of determination (r^2) values indicated that only minimum dissolved oxygen (r^2 = 75.3 %) and maximum pH

Table 2. Relation of selected water quality variables to mean mortality of caged prickly sculpin exposed for 24-hours to ambient water quality conditions in Rodeo Lagoon and Rodeo Lake. N, sample size; r, Pearson correlation coefficient; P, probability.

		Mean mortality	
Water quality variable	N	r	P
Maximum temperature	36	0.1040	0.5461
Minimum dissolved oxygen	36	-0.8682	<0.0001
Maximum pH	36	-0.7271	<0.0001
Maximum salinity	36	-0.5816	0.0002
Maximum turbidity	36	-0.4450	0.0065
Maximum un-ionized ammonia	36	-0.6904	<0.0001

(r^2 = 52.9 %) accounted for over half of the variation in fish mortality.

When 3-dimensional plots of the data from all tests were examined, mortality of prickly sculpin exhibited little or no relationship with maximum temperature, maximum pH, maximum salinity, maximum turbidity, and maximum un-ionized ammonia (Fig. 2a-2e). Although some tests yielded high (>70 %) mortality at temperatures of 14.2-14.5°C, pH values of 7.0-7.3, salinity concentrations of 0.4 ‰, turbidity levels of 13.0-24.5 NTUs, and un-ionized ammonia concentrations of 0.0002-0.0004 mg/L, other tests yielded little or no mortality under similar or more extreme conditions. On the other hand, high mortality (>70 %) occurred whenever dissolved oxygen concentrations dropped to 3.80 mg/L, whereas mortality was low (0 %) when dissolved oxygen concentrations equaled or exceeded 4.56 mg/L.

When water quality variables (main effects) were examined by stepwise logistic regression, the large Wald chi-square statistic associated with minimum dissolved oxygen concentration indicated that this variable was the most important predictor of fish mortality (Wald χ^2 score=25.1, P<0.0001). However, maximum pH (Wald χ^2 score=5.4, P=0.0204) also accounted for a significant amount of variation in fish

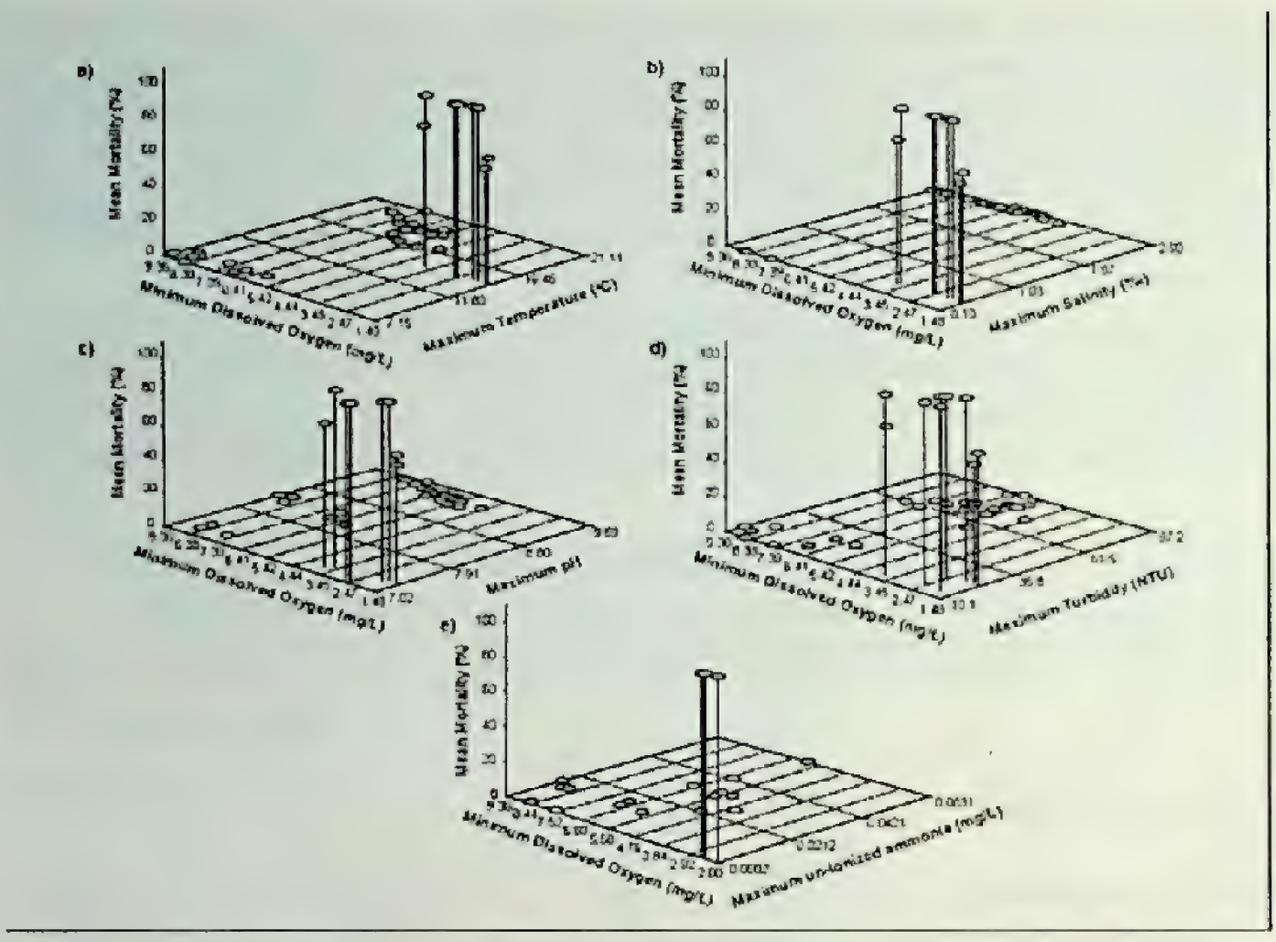


Fig. 2. Three-dimensional plots of mean mortality in prickly sculpin exposed for 24 hours to selected water quality variables in Rodeo Lagoon and Rodeo Lake over all tests: (a) minimum dissolved oxygen concentration and maximum temperature; (b) minimum dissolved oxygen concentration and maximum salinity; (c) minimum dissolved oxygen concentration and maximum pH; (d) minimum dissolved oxygen concentration and maximum turbidity; and (e) minimum dissolved oxygen concentration and maximum un-ionized ammonia concentration.

mortality.

DISCUSSION

Our search of the published literature yielded little information on the tolerance of prickly sculpin to water quality variables. Black (1953) reported that prickly sculpin acclimated to 18-19°C experienced no mortality when held for 24 hours at a temperature of 22.8°C, 50% mortality when held at 24.1°C, and 100% mortality when held at 26.5°C. However, experiments conducted by Bond (1963) indicated that prickly sculpin suffered mortalities only when temperatures approached or exceeded 30.2°C. By comparison, the maximum temperature that we recorded in Rodeo Lagoon and Rodeo Lake was 21.1°C. These observations suggest that temperature alone was probably not sufficiently elevated to cause the mortalities observed during our study.

According to Bond (1963), prickly sculpin died within 24 hours when exposed in

the laboratory to dissolved oxygen concentrations of 1.24 mg/L or less, with low mortality occurring at dissolved oxygen concentrations of 1.9 mg/L and higher. However, Bond also noted that his laboratory findings failed to account for the distribution of prickly sculpin along dissolved oxygen gradients in the Willamette River drainage, Oregon, because this species was not captured from several reaches where dissolved oxygen minima were as high as 4 mg/L. By comparison, low concentrations of dissolved oxygen accounted for most of the variation in mortality of prickly sculpin during our study, with high mortality (>70 %) occurring when fish were exposed to dissolved oxygen concentrations of 3.8 mg/L or less, and no mortality occurring at dissolved oxygen concentrations above 4.5 mg/L. The apparent discrepancy between Bond's laboratory findings and both his and our field observations raise the possibility that laboratory results may not always extrapolate to field conditions. This conjecture is supported by Parrish (1985), who noted that oxygen requirements of fish held in the laboratory (where they typically exhibit low activity levels) can be less than those of fish living in the natural environment.

The prickly sculpin is well known for its euryhaline ability which allows it to inhabit both freshwater and brackish environments (Krejsa 1967; Moyle 2002). According to Bond (1963), the 96-hour median tolerance limit of juvenile prickly sculpins to salinity was 27.5‰, whereas the 96-hour median tolerance limit of adults was 30‰. However, Herbold (2000[7]) mentioned that prickly sculpins are seldom found in salinities greater than 10‰. Salinities did not exceed 2.9‰ at our test sites, suggesting that this variable was not directly responsible for high mortality of prickly sculpin recorded during the fall in Rodeo Lake.

To our knowledge, published data are not available on the tolerances of prickly sculpin to other water quality variables (pH, turbidity, and un-ionized ammonia concentration) that we measured in Rodeo Lagoon and Rodeo Lake. Our finding that maximum pH values were inversely correlated with mortality of prickly sculpin was most likely a statistical artifact created by low dissolved oxygen concentrations (≤ 3.8 mg/L) occurring at the lower end of the pH range (pH 6.98-7.31) measured during our study (see Fig. 2c). In general, circumneutral pH values are considered harmless to most fish species, with USEPA (1986) recommending that pH not exceed 6.5-9.0 to protect freshwater aquatic life and 6.5-8.5 to protect marine aquatic life. However, even within these pH ranges, the toxicity of total ammonia can increase in response to increasing pH due to a chemical equilibrium shift that causes the un-ionized form of ammonia to predominate (Russo 1985). Although not documented during our study, fish can be harmed by exposure to pH values that exceed the USEPA criteria. For example, Serafy and Harrell (1993) noted that high pH might adversely affect the proper functioning of

[7]Herbold, B. 2000. Prickly sculpin *Cottus asper*. pp. 126-128 *In*: P.R. Olofson (ed.). Baylands ecosystem species and community profiles: life histories and environmental requirements of key plants, fish and wildlife. Prepared by the San Francisco Bay Area Wetlands Ecosystem Goals Project, San Francisco Bay Regional Water Quality Control Board, Oakland, California.

fish gills (by damaging surface tissues and precipitating excessive mucous secretion) and severely impair oxygen uptake.

Other unmeasured variables associated with algal blooms may also influence fish mortality. According to Carmichael and Gorham (1981), *Anabaena* and *Microcystis*—two genera of blue-green algae occurring under bloom conditions in Rodeo Lagoon—can produce toxins that are potentially lethal to fish. In July 2006, four different microcystins were detected in two of four water samples collected from the lagoon (Ramos 2006[8]). However, fish mortalities were not observed at the time that the water samples were collected.

In summary, findings from our study indicate that temporary depletion of dissolved oxygen may be the primary reason that prickly sculpin and other fishes experience occasional die-offs during the fall in Rodeo Lagoon and Rodeo Lake. The most likely explanation is that excessive decomposition of plant material during the senescence phase of algal blooms or diebacks of rooted macrophytes depletes dissolved oxygen from the water column, resulting in hypoxia and asphyxiation of fish. During one previous fish die-off in Rodeo Lagoon, Fong (1997[1]) observed sculpins swimming at the surface in shallow water, a behavior not typically observed in this species. Fong's observations are consistent with aberrant behavior described in fish experiencing severe oxygen depletion wherein still-living individuals attempt "to gulp air" (Herman and Meyer 1990).

ACKNOWLEDGMENTS

We thank L. Canevaro, A. Hui, E. Mahl, and L. Ono for assisting with field work.

LITERATURE CITED

Black, E.C. 1953. Upper lethal temperatures of some British Columbia freshwater fishes. Journal of the Fisheries Research Board of Canada 10:196-210.

Bond, C.E. 1963. Distribution and ecology of freshwater sculpins genus *Cottus*, in Oregon. Ph.D. dissertation, University of Michigan, Ann Arbor, Michigan, USA.

Carmichael, W.W. and P.R. Gorham. 1981. The mosaic nature of toxic blooms of Cyanobacteria. Pages 161-172 *in*: W.W. Carmichael, editor. The water environment: algal toxins and health. Plenum, New York, USA.

Emerson, K., R.C. Russo, R.E. Lund, and R.V. Thurston. 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. Journal of the Fisheries Research Board of Canada 32:2379-2383.

Herman, R.L., and F.P. Meyer. 1990. Fish kills due to natural causes. Pages 41-44 *in*: F.P. Meyer and L.A. Barclay, editors. Field manual for the investigation of fish kills. U.S. Fish and Wildlife Service, Resources Publication 177, Washington, DC, USA.

Krejsa, R.J. 1967. The systematics of the prickly sculpin, *Cottus asper* Richardson, a polytypic

[8]Ramos, M. 2006. Final Report for accession #D0608059. California Animal Health & Food Safety Laboratory System (CAHFS)-Davis, unpublished final report, 6 p.

species. Part I. Synonymy, nomenclatural history, and distribution. Pacific Science 21:241-251.

Martin, B.A., and M.K. Saiki. 1999. Effects of ambient water quality on the endangered Lost River sucker in Upper Klamath Lake, Oregon. Transactions of the American Fisheries Society 128:953-961.

McKee, J.E., and H.W. Wolf, editors. 1963. Water quality criteria; second edition. Publication No. 3-A, California State Water Resources Control Board, Sacramento, CA, USA.

Moyle, P.B. 2002. Inland fishes of California. University of California Press. Berkeley, California, USA.

Parrish, P.R. 1985. Acute Toxicity Tests. Pages 31-57 *in*: G.M. Rand and S.R. Petrocelli, editors. Fundamentals of aquatic toxicology, Taylor and Francis, Washington, D.C. USA.

Russo, R.C. 1985. Ammonia, nitrite, and nitrate. Pages 455-471 *in*: G.M. Rand and S.R. Petrocelli, editors. Fundamentals of aquatic toxicology; methods and applications. Taylor and Francis (Hemisphere Publishing Corporation), Bristol, Pennsylvania, USA.

Serafy, J.E., and R.M. Harrell. 1993. Behavioural response of fishes to increasing pH and dissolved oxygen: field and laboratory observations. Freshwater Biology 30:983-988.

USEPA (U.S. Environmental Protection Agency). 1986. Quality criteria for water; 1986. EPA 440/5-86-001, U.S. Government Printing Office, Washington, DC, USA.

Walters, G.L. 1989. Hach water analysis handbook. Hach Company, Loveland, Colorado, USA.

Received: 30 March 2006
Accepted: 18 November 2006

California Fish and Game 93(4):224-227 2007

INCONSISTENCIES IN HISTORICAL GEOGRAPHIC RANGE MAPS: THE GRAY WOLF AS EXAMPLE

STEPHANIE L. SHELTON[1] AND FLOYD W. WECKERLY
Department of Biology
Texas State University
San Marcos, TX 78666
Correspondent email: stephanie.shelton@gmail.com

Range maps depicting historical distributions of wildlife may be inconsistent. Different maps can be based on diverse sources of evidence which may vary in reliability (e.g., specimens in Natural History Museums, trapper and hunter journals, conversations recorded in dairies) and the effort expended locating evidence may differ among map makers (Young and Goldman 1944, Seton 1953, Hall 1981). Despite these limitations, maps depicting historical distributions are useful to individuals and institutions concerned with maintenance of biodiversity or restoration of native species to areas where they were extirpated. In this note, we used maps of the historical distribution of the gray wolf, *Canis lupus*, to exemplify such inconsistencies.

Once found throughout much of North America, gray wolf populations within the contiguous United States were almost extirpated, but some populations in Canada, Alaska, and Mexico have remained largely intact (Young and Goldman 1944, Leopold et al. 1981). Similar situations exist with other mammalian species in the United States, particularly large, charismatic herbivores and carnivores such as bison, *Bos bison*, elk, *Cervus elaphus*, mountain sheep, *Ovis canadensis*, and grizzly bear, *Ursus arctos* (Hall 1981). Available historical distribution data can assist in restoration efforts for these mammalian species in particular.

MATERIALS AND METHODS

We selected historical range maps of North American gray wolves that were developed independently of one another (Fig. 1). Historical was defined as the time period around 1500, the time before extensive colonization by Europeans. We considered distribution maps to be independent if the authors did not state their distribution maps were based on findings from other studies. We used range maps from (Fig. 1) Young and Goldman (1944), Seton (1953), Hall (1981), and Nowak (2002). The chosen sources present their gray wolf range maps as common knowledge of the distribution of the gray wolf in North America. No explicit details on how these maps were created appear in any of the sources.

Each independent North American source range map was overlaid onto a base map of the continental United States by heads up digitization using ArcMap 9.0 (Environmental Research Institute, 2004). This final ranked map depicted the agreement and differences among the four maps in the historical range distribution of the gray wolf in the United States. Rankings were shown on the final map from 0-4. Areas with 0

[1]Current address: Stephanie Shelton, 12610 Live Oak Lane, Buda, TX 78610

indicated all source maps showed the absence of the gray wolf, whereas areas with 4 represent all source maps showed the historical presence of the gray wolf.

Hall, E. R. 1981. The mammals of North America.

Nowak, R. M. 2002. The original status of wolves in eastern North America.

Young, S. P. and E. A. Goldman. 1944. The wolves of North America: Part I - their history, life habits, economic status, and control.

Seton, E. T. 1953. Lives of game animals, Vol. 1: cats, wolves, & foxes.

Fig. 1 Historical gray wolf range maps obtained from available literature. The grey area on each source range map denotes gray wolf distribution according to the author.

RESULTS

A majority of the gray wolf historical range where the four maps are consistent occurs in the northern, central, and northwestern United States, inconsistencies are in the western and southeastern portions of the country (Fig. 2). The southeastern portion of the United States follows a pattern of agreement among maps ranging from four to two from North to South, respectively. Three or more maps agreed in the Northeast, but agreement decreased in the east-central part of the country in New Jersey, Pennsylvania, Ohio, Indiana, Illinois, Missouri, Oklahoma, and Texas.

Disagreement among source range maps is most pronounced in California and Arizona, followed by Washington, Oregon, Utah, and Nevada (Fig. 2). Small parts of Idaho and Wyoming have inconsistencies ranging from four to two maps in agreement. Part of California and a small portion of southwest Arizona are the only two states with rankings of 1, meaning only one of the maps suggests the historical presence of gray wolves.

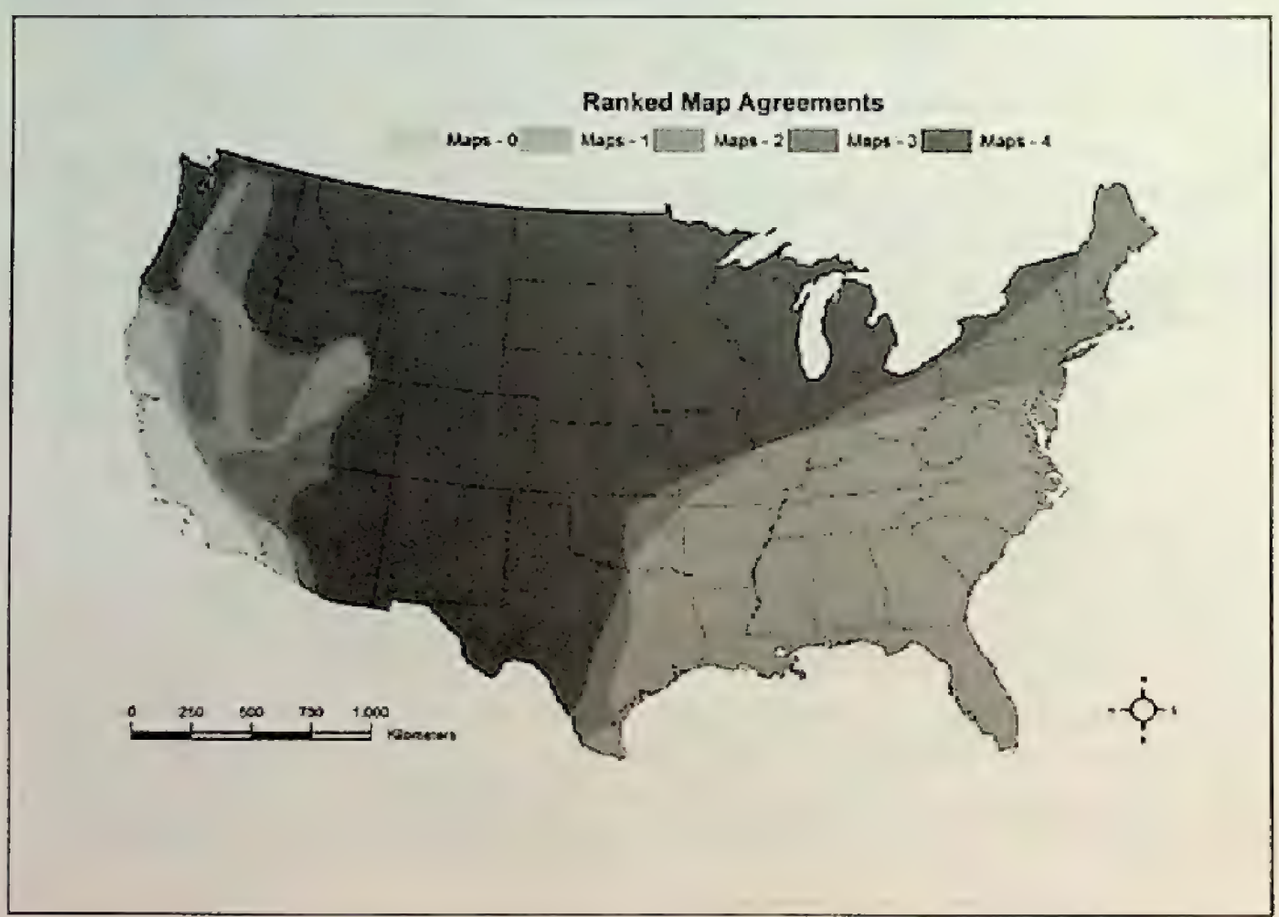


Fig. 2 The ranked map agreements for the presence of the gray wolf in the United States based on four independent historical range maps.

DISCUSSION

Correspondence of range maps in the southeastern states was lacking, probably due to unresolved species relationships among the gray wolf, coyote, *Canis latrans*, and red wolf, *Canis rufus* (Nowak 2002). Most discrepancies among historical gray wolf range maps occurred in the western states, especially in California. Range maps used in our analysis indicate gray wolves rarely occupied the central, coastal, or southern portions of the state. Young and Goldman (1944) postulated that wolves were rarely found in deserts; but some of their results indicated that gray wolves did inhabit those regions. Early records document wolves in the Sacramento Valley, and near the San Joaquin River in Madera County (Young and Goldman 1944). In 1918, a wolf was killed in Los Angeles County and in 1922 a gray wolf was trapped in the Providence Mountains, San Bernardino County (Young and Goldman 1944:58, Hall 1981). Young and Goldman (1944) and Hall (1981) report wolves probably inhabited areas near Mono Lake and Mount Dana in Mono County in 1930.

Information for many historical range maps came from diaries of trappers, settlers, and explorers. Some range boundaries may be questionable because they were based on historical records or erroneous descriptions of specimen locations. Revisiting original records of gray wolf distribution may help to produce more meaningful and, perhaps, accurate range maps but many descriptions are cursory or may reflect inaccurate location data. Schmidt (1991:84) suggested that further research of artifacts, historical documents, and other "paleontological searches" be conducted to enhance data already available. Differences among historical range maps of the gray wolf suggests more effort is needed to identify historical animal ranges in the western United States, particularly in California, the only state where, apparently, large areas were never occupied by wolves.

Failure to confirm animal sightings or obtain additional sources of evidence to corroborate historical presence of wildlife may lead to inconsistencies in historical range maps. Our analysis of historical range maps of the gray wolf illustrates this. It is likely that historical range maps of other charismatic, large mammal species are also inconsistent.

LITERATURE CITED

Hall, E. R. 1981. The mammals of North America. John Wiley & Sons, New York, USA.

Leopold, A. S., R. J. Gutierrez, and M. T. Bronson. 1981. North American game birds and mammals. Charles Scribner's Sons, New York, USA.

Nowak, R. M. 2002. The original status of wolves in eastern North America. Southeastern Naturalist 1:95-130.

Schmidt, R. H. 1991. Gray wolves in California: their presence and absence. California Fish and Game 77:79-85.

Seton, E. T. 1953. Lives of game animals. Charles T. Branford Co., Boston, USA.

Young, S. P. and E. A. Goldman. 1944. The wolves of North America: Part I – their history, life habits, economic status, and control. The American Wildlife Institute, Washington D. C., USA.

Received: 27 August 2006
Accepted: 24 February 2007

REFEREE ACKNOWLEDGMENTS

The following individuals have reviewed manuscripts for *California Fish and Game* during 2007. The list is as complete as possible. The editors apologize for any omissions and sincerely thank all those who have contributed to the quality of the journal by providing competent and timely reviews.

Allen, L.G.
Ballock, W.
Bartholomew, J.
Baskin, J.N.
Beer, W.
Bradley, J.
Chen, Y.
Cox, B.
Crayon, J.
Fabula, T.
Fleming, K.
Hayes, C.
Horn, M.
Kashiwada, J.
Love, C.
Love, M.
Macewicz, B.J.
Matern, S.
Maule, A.
McKiver, D.
Monteith, K.
Murie, D.
Roberts, E.
Saiki, M.
Schlosser, S.
Scoppettone, G.
Shaffer, K.
Swift, C.
Villepique, J.
Walker, H.J.
Watters, D.
Wehausen, J.

California Fish and Game 93(4):228-235 2007

INDEX TO VOLUME 93 (2007)

AUTHORS

SUBJECT

SCIENTIFIC NAMES

OSP 07 105191

INSTRUCTIONS FOR AUTHORS

California Fish and Game is a professional, scientific journal devoted to the conservation and understanding of California's flora and fauna. Original manuscripts dealing with California species or providing information of direct interest and benefit to California researchers and managers are welcome.

MANUSCRIPTS: Refer to the CBE Style Manual (6th Edition) and a recent issue of *California Fish and Game* for general guidance in preparing manuscripts. Specific guidelines are available in *California Fish and Game* 87(2):77-85.

COPY: Use good quality 215 x 280-mm paper. Double-space throughout with 25-mm margins. Do not hyphenate at the right margin or right-justify text. Authors should submit four good copies of their manuscript, including tables and figures, to the Co-Editors-in-Chief. An electronic copy of the manuscript on diskette in word processor format will be required with the final accepted version.

CITATIONS: All citations should follow the name-and-year system. See a recent issue of *California Fish and Game* for the format of citations and Literature Cited. Completely spell out publication and periodical names in Literature Cited. Avoid references to unpublished literature.

ABSTRACTS: Every article, except notes, must be introduced by an abstract. Abstracts should be about one typed line per typed page of text. In one paragraph describe the problem studied, most important findings, and implications of the results.

TABLES: Start each table on a separate page and double-space throughout. Do not use vertical rules. Use tabs, not the spacebar, to space between columns. Footnotes in tables should be consecutive lower-case letters, with the sequence beginning again in each table.

FIGURES: Consider proportions of figures in relation to the usable page size of *California Fish and Game* (117 x 186 mm). Figures, including captions, cannot exceed this size. Figures and line-drawings should be clear, with well-defined lines and lettering. Lettering style should be the same throughout and large enough to be readable when reduced to finished size. Type figure captions on a separate page. High-quality photographs with strong contrast are acceptable and should be submitted on glossy paper. On the back and top of each figure or photograph, lightly write the figure number and senior author's last name. Be prepared to provide high-quality, scannable, original figures or graphics files on diskette with the final accepted manuscript.

PAGE CHARGES AND REPRINTS: All authors will be charged $40 per printed page and will be billed before publication of the manuscript. Explicit acceptance of page charges should be included in the submittal letter. Authors will receive a reprint order form along with the galley proof.

Editor, ***CALIFORNIA FISH AND GAME***
California Department of Fish and Game
PO Box 944209
Sacramento CA 94244-2090